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FACTORS INDUCING MINERAL-DEFICIENCY SYMPTOMS ON THE POTATO PLANT

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(With 1 Text-figure)

Increased attention has been given in recent years to the study of nutrient-deficiency symptoms in different plants. The published results of this type of work have been derived from examination of the plant grown more often in sand or water culture than in the field. A plant grown in sand culture and in the field may react differently to the same deficiency owing to variation in the cultural conditions, since it is impossible to reproduce in sand the complexity of factors present in soil, the interactions of which may induce not only the incidence but also the nature of deficiency symptoms. The study of plants grown under controlled conditions in sand and water culture may assist the diagnosis of deficiency symptoms under field conditions, but extreme care is necessary in interpreting the results so obtained in terms of the behaviour of the plants under natural conditions.

The external reactions of a plant to deficiencies or to sufficiencies of plant foods may afford a better understanding of the mutual relationship between soil and plant than even an elaborate soil analysis. Field studies of this type would at least facilitate interpretation of soil analyses and thus contribute to the solution of manurial problems. In this connexion there may be grounds for regret that modern field fertilizer trials, in which the responses can be assessed statistically, have not been more utilized for the study of the incidence of nutrient deficiencies in relation to manurial treatments. The evidence from previous studies suggests that the effects of a nutrient deficiency on the plant may be intensified by some specific interaction between the deficient element and one or more of the other elements. Similarly, the presence of one or more nutrients in definite excess in the soil may interfere with the solubility, absorption or utilization of another element to such a degree as to induce acute deficiency symptoms on the plant, although there might be an adequate supply of the second element for normal growth in the absence of the excess of other elements.

A comprehensive publication (Hambidge, 1941), illustrated by colour and black and white plates, deals with deficiency symptoms on a wide range of plants. A similarly illustrated publication (Eckstein *et al.* 1937) is devoted exclusively to potash-deficiency symptoms. Potash-deficiency effects, as exhibited by the potato plant in the field, are well illustrated in both these publications. It has been generally assumed that leaf scorch and other potash-deficiency symptoms are primarily induced by a combination of high nitrogen and low potash in the soil. The abnormally green colour of the foliage on the NP plots was attributed mainly to the unbalancing influence of the nitrogen. The assumption was based on observations of plots in the older type of experiment which did not usually include separate nitrogen and phosphate treatments. Wallace (1925 *a*, *b*) showed that a wide ratio of nitrogen to potash (nitrogen/potash) was a vital factor in the incidence of leaf scorch on fruit plants. Findlay (1928) and Tottingham *et al.* (1936) have adduced blackening of cooked potatoes as a symptom of potash deficiency.

Phosphate-deficiency symptoms of the potato plant grown in the field have not previously been described in detail. Jones & Brown (see Hambidge, 1941) refer to the effect of phosphate deficiency

(NK manuring) in slowing down initial growth and prolonging growth in the autumn. Rusty-brown lesions, as isolated flecks tending to fuse together, have been found on tubers grown on phosphate-deficient soil. Van Shreven (1935) has found in sand culture that phosphate-deficient potato plants are somewhat rigid and the leaflets smaller and darker than normal, with petioles, leaflets and leaf margins tending to turn upwards.

Calcium-deficiency symptoms relating to the potato plant in the field have not been reported in the literature. This is surprising as one would naturally expect to find calcium-deficiency symptoms in the potato plant on acid soils under heavy rainfall conditions.

Magnesia deficiency in the potato is reported from practically all the potato districts in the Atlantic Coastal Plain in the U.S.A. It generally occurs on acid sandy soils subject to heavy leaching by rain. Magnesia deficiency commences by paleness of the tips and margins of the bottom leaves and progresses between the vein towards the centre of the leaflet. In advanced stages of deficiency the interveinal tissue in the centre of the leaflet becomes chlorotic and is eventually characterized by small brown necrotic areas. The terminal leaflet is generally the one most severely affected.

Manganese deficiency in the potato plant is indicated by paleness of the tip of the foliage accompanied by dark brown interveinal spots. Morley Davies (1939) found it liable to occur on soils with reaction values above 6·5 *p*H combined with a high organic matter content. Manganese deficiency sometimes occurs on acid sandy soils owing to loss of soluble manganese by leaching.

Boron deficiency is most frequently associated with alkaline soil conditions in which the soil boron has become immobile. It is generally more prevalent in dry years. O'Brien & Dennis (1936) found that a borax application reduced the incidence of a non-parasitic leaf mould in var. Gladstone and of an internal rust-spot condition in var. Golden Wonder. Smith & Nash (1937) found that borax reduced sloughing (falling away of outer layer) of cooked tubers, increased flouriness and dryness, and improved texture, flavour and colour.

Iron deficiency has not been reported in relation to the potato plant in the field. In acid soils on which potatoes are usually grown there is generally sufficient iron in the soil solution to cover growth requirements. Malnutritional chlorosis due to deficiency of available iron usually occurs on calcareous soils.

Experimental

An opportunity for studying certain mineral-deficiency symptoms of the potato plant was presented by two large series of replicated field trials carried out by the writer in 1937 and 1938 respectively in association with Dr E. M. Crowther of Rothamsted Experimental Station. These experiments were designed to examine the main effects of phosphates, potash and magnesia and their interactions, but they were well adapted to the intensive study of mineral-deficiency symptoms by reason of the low fertility of most of the soils tested and the large number of direct comparisons between mineral treatment and no mineral treatment afforded by each experiment.

The experimental design adopted in 1937 was the $3 \times 3 \times 3$ single replication, involving three randomized blocks of nine plots each and all combinations of three levels of the fertilizers, the error being estimated from higher order interactions. A basal dressing of nitrogen equivalent to 0·6 cwt. nitrogen/acre in the form of sulphate of ammonia was applied equally to all the plots. The levels of phosphates, potash and magnesia tested per acre, in the form of superphosphate, sulphate of potash and magnesia, were (1) nil, (2) 0·5 cwt. phosphoric acid, (3) 1·0 cwt. phosphoric acid; (1) nil, (2) 0·7 cwt. K_2O, (3) 1·4 cwt. potash (K_2O); (1) nil, (2) 0·3 cwt. MgO, (3) 0·6 cwt. MgO.

In 1938 the factorial design $2 \times 2 \times 2 \times 2$ was used, involving two randomized blocks of eight plots each (plus one untreated) set down in duplicate and testing all combinations of two levels of the fertilizers, third order interactions being confounded with block differences. The treatments tested per acre were the following: (1) 0·3 cwt. N, (2) 0·6 cwt. N, as sulphate of ammonia; (1) 0, (2) 1 cwt. P_2O_5 as superphosphate; (1) 0, (2) 1·4 cwt. K_2O as sulphate of potash; (1) 0, (2) 0·6 cwt. MgO as sulphate of magnesia.

The experiments were carried out mostly on acid sandy soils of low fertility, with *p*H values ranging between 4·0 and 6·8 and without the addition of dung, for the purpose of securing as large responses as possible to the minerals to be treated. The experimental centres were widely spread from north of

Scotland to south of England and included soil types as widely divergent as the following: bunter sandstone, Bagshot sands, lower greensands, Keuper sandstones, granite soils, glacial sands and fen soils. The total number of trials involved was 24 in 1937, and 25 in 1938. With four exceptions in each season var. Majestic was grown in the trials.

Field observations

Potash deficiency

Observations showed that potash-deficiency symptoms, wherever they occurred, were consistently the most marked on the plots receiving both nitrogen and phosphates. At some centres these symptoms were so pronounced as to render the NP plots strikingly conspicuous in the experimental areas. The successive stages of potash deficiency noted on NP plots might be summarized as follows: (1) dark green succulent foliage from earliest stage, the upper surfaces of the leaflets being markedly crinkled and glossy, sometimes with a greyish tint suggestive of 'silver-leaf'; (2) development of a squat habit of growth caused by short internodes and bending of the main stems towards the ground; (3) browning or 'coppering' of the interveinal leaf tissue; (4) marginal leaf scorch; (5) blackening and early death of the haulms. It was noted that leaf scorch developed rapidly during drought and the plants always showed some recovery after rain. The symptoms were consistent in character over the different experimental centres, although they obviously varied in intensity according to the potash status of the soils. The symptoms were markedly intensified by increasing the nitrogen level in the nitrogen-phosphate treatment (N_2P), but not by increasing the phosphates (NP_2).

The plots receiving nitrogen only, either at the lower or higher level (N_1 and N_2), showed no marked symptoms of potash deficiency, even on low-potash areas, apart from a few centres where the symptoms were nearly as acute as those of the NP plants. The nitrogen plants, although darker green and smaller than the KPN plants, appeared to grow normally and died off similarly to, but sometimes later than, the latter plants, with natural yellowing of the leaves from the bottom upwards.

The above observations indicated that phosphates, in association with nitrogen, play an essential part in developing potash-deficiency symptoms on the potato plant under field conditions.

Discussion. Light was thrown upon the difference between nitrogen and NP treatment effects in relation to the occurrence of potash-deficiency symptoms of the potato plant by a chemical investigation undertaken by Knowles *et al.* (1940) into the nutrient uptake of the potato plant under different manurial conditions in the field. The soil involved in this investigation was an acid drift gravel of low nutrient status at Bromley, Essex. The results indicated that nitrogen markedly increased the absorption of potash by the plant, which explained the absence or reduced incidence of potash-deficiency symptoms associated with nitrogen treatment. On the other hand, nitrogen and phosphates together severely reduced the uptake of potash, and this adverse effect was the more pronounced the higher the level of nitrogen in the combined treatment (N_2P).

The data further indicated that NP manuring induced not only a more rapid attainment of maximum potash uptake but an earlier completion of the migration of potash from haulms to tubers as compared with nitrogen or NPK treatment. Phosphates alone had only a slight effect on potash absorption. This result agreed with the relatively small incidence of potash-deficiency symptoms observed on the phosphorus plots. The cumulative evidence thus led to the conclusion that some interaction between nitrogen and phosphates was

responsible for the diminished uptake of potash and for the occurrence of acute potash-deficiency symptoms induced by this type of manuring. The mechanism of this action was not revealed by the chemical data, although somewhat larger than normal concentrations of nitrogen and phosphates found in the roots of the young plants may have had some inhibiting effect on the absorption of potash.

Further investigations revealed the reasons for the occurrence of potash-deficiency symptoms on the nitrogen plots in a comparatively small number of the field trials. The twenty-two centres at which marked potash-deficiency symptoms appeared were examined and classified into two groups: (*a*) fifteen centres where little or no leaf scorch appeared on the nitrogen plots, and (*b*) seven centres where leaf scorch was marked on the nitrogen plots. The complete data, including information on the phosphate status of the soils at the respective centres in each group, are given in Table 1. Fig. 1 reveals the relationship between the various factors: (1) the amounts of readily-soluble phosphates in the soil, (2) the actual responses to superphosphate, and (3) the occurrence or absence of leaf scorch on the nitrogen plots.

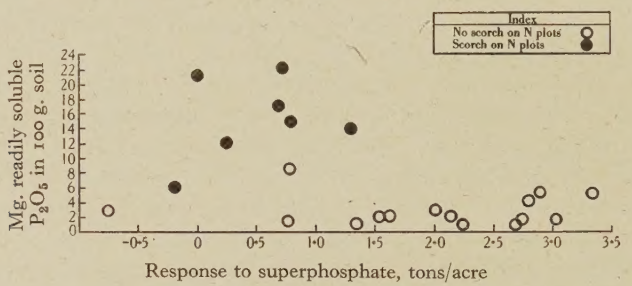

Fig. 1. Relation between presence or absence of leaf scorch on the N plots and the phosphate status of the soil—1937 and 1938.

The results indicate that the incidence of leaf scorch on the nitrogen plants is determined by the phosphate status of the soil. The centres, with one or two divergencies in each group, fall closely into two distinct areas in Fig. 1, demonstrating the absence or small incidence of leaf scorch on the nitrogen plots when the phosphate status of the soil is low, and the occurrence of leaf scorch on the nitrogen plots in conjunction with a relatively high level of available phosphates in the soil. These results are in accordance with the observations made that leaf scorch consistently appeared on the NP plots on soils with low potash reserves. The differential response shown by the potato plant to nitrogen and NP treatments on soils of varying mineral status throws light upon the relative levels of available phosphates and potash in the soil. The depressing influence of an excess of nitrogen and phosphates on potash absorption is of importance in relation to low potash soils. Where it is not feasible in such cases to raise the level of available potash in the soil, through the prevailing shortage of potash fertilizers, the conditions may be ameliorated by reduction or omission of phosphate applications.

The symptoms associated with potash deficiency as reflected by the tubers were also studied. These might be classified as follows: (*a*) smaller size of the potato tubers as shown by the larger percentage of non-ware or non-marketable tubers: this result was characteristic

TABLE I

Centre	Soil	*p*H	m.g. P_2O_5/100 g. soil*	Mean response to super-phosphate tons/acre	S.E.	Mean yield tons/acre	NP plots	N plots
Group A. 15 centres—no marked leaf scorch on N plots								
Liss, Hampshire	Lower greensand (podzolized)	4·1	1	1·36†	±0·36	7·86	Scorch	No scorch
Alvington, Gloucestershire	Light sandstone (old red sandstone)	5·1	1	2·72†	±0·74	11·96	,,	,,
Liskeard, Cornwall	Light loam (granite)	5·5	1	2·27†	±0·28	5·51	,,	,,
Cheswardine, Shropshire	Sandy loam (bunter sandstone)	5·0	2	1·67†	±0·31	10·95	,,	,,
Dymock, Gloucestershire	Light sand (old red sandstone)	5·8	4	2·82†	±0·59	12·48	,,	,,
Hinton, Hampshire	Light soil (Bagshot sands)	4·9	1	0·79	±0·53	8·06	,,	,,
Hednesford (1937), Staffordshire	Sandy loam (bunter sandstone)	4·8	2	2·78†	±0·32	2·95	,,	,,
Stanton-on-Hine, Heath, Shropshire	Sandy loam (bunter sandstone)	4·8	3	−0·26	±0·63	5·17	,,	,,
Moretonhampstead, Devon	Light loam (granite)	4·8	3	2·01†	±0·59	8·17	,,	,,
Upton, Dorset	Light soil (Bagshot sands)	4·9	2	2·13†	±0·23	6·97	,,	,,
Redmarley, Gloucestershire	Light sand (old red sandstone)	5·4	9	0·78	±0·49	13·09	,,	,,
Lustleigh, Devon	Light loam (granite)	4·8	5	3·36†	±0·20	7·04	,,	,,
Pittensair, Morayshire	Light sand (glacial)	4·9	5	2·88†	±0·21	6·95	,,	,,
Hednesford (1938), Staffordshire	Sandy loam (bunter sandstone)	4·7	2	3·01†	±0·21	5·11	,,	,,
Tunbridge Wells, Kent	Light loam (Hastings beds)	5·3	2	1·51†	±0·48	13·19	,,	,,
		Mean	3	1·99				
Group B. 7 centres—marked leaf scorch on N plots								
Lhanbryde, Morayshire	Light sand (glacial)	5·4	15	0·90‡	±0·33	8·99	,,	Scorch
Tholthorpe (1937), Yorkshire	Light sand (glacial)	4·9	12	0·27	±0·15	4·50	,,	,,
Caerwent, Monmouthshire	Light loam (old red sandstone)	6·8	6	−0·17	±0·40	5·11	,,	,,
Bedlington, Northumberland	Boulder clay on coal measures	6·2	14	1·26‡	±0·17	10·07	,,	,,
Tholthorpe (1938), Yorkshire	Light sand (glacial)	5·0	22	0·71	±0·27	8·33	,,	,,
Drem, East Lothian	Medium loam	5·7	17	0·68	±0·37	14·70	,,	,,
Rugeley, Staffordshire	Sandy loam (bunter sandstone)	5·3	21	0·02	±0·55	8·19	,,	,,
		Mean	15	0·52				

* Readily soluble phosphorus by acetic acid or by modified Morgan's method.
† = 1 % significance. ‡ = 5 % significance.

of both nitrogen and NP treatments; (*b*) malformation or pear-shaped contour of the tubers in acute cases of potash deficiency resulting from NP treatment: this shape was caused by the tapering of the potato towards the stem end of the tuber or the end attached to the parent plant; (*c*) blackening and 'soapy' texture of the cooked tubers, characteristic of both nitrogen and NP treatments. The mechanism of this process of blackening has not been elucidated, but as the change is induced by both nitrogen and NP treatments, the factors involved would appear to be different from those causing the leaf symptoms of potash deficiency which are attributable only to NP effects. The observation that tubers from plants grown without fertilizer showed no marked tendency to blacken after cooking suggests that this feature is related to an ill-balanced nutritional condition rather than to a general under-nourishment of the plant.

Phosphate deficiency

The experiments also provided opportunities for studying phosphate-deficiency symptoms on the potato plant under conditions in the field. Table 1 shows that at thirteen centres large positive responses to phosphate applications were obtained. These responses were associated with soils of different types, evidently of low phosphate status as also indicated, with one exception, by the figures representing the readily soluble phosphate in the soil. The high responsiveness of these particular soils to phosphate dressings was probably due to several factors—the naturally low mineral status of sandy soils, the crop removal of phosphates without adequate replacement, and the occurrence of high phosphate fixation in the more acid soils, as suggested by the low *p*H values. In connexion with the latter factor, it is significant that nine out of the fifteen soils in group A, with high phosphate requirements, had *p*H values below 5, as compared with only one out of seven soils in group B, where the levels of available phosphate are generally higher.

The omission of phosphate applications at twelve centres in group A resulted not only in considerably lower yields, but in distinctive growth and foliar symptoms of the plants. The characteristic symptoms, observed in association with phosphate deficiency, were (1) delayed emergence of the plants and slower growth especially in the early stages, (2) dull green or bronzing of upper surfaces of the leaves, (3) rigid and spindly growth with meagre branching, (4) tendency of leaves to grow upwards forming acute angles with the stems, (5) upward curling of the margins of the leaflets, and (6) late dying down of haulms. These symptoms appeared to be accentuated more by NK than by nitrogen treatment. It was also consistently noted that under conditions of low phosphates and low potash in the soil the nitrogen plants exhibited phosphate-deficiency and not potash-deficiency symptoms. Phosphate deficiency, as exemplified by NK treatment, was further reflected by the immature appearance of the tubers and a notable lack of flouriness after cooking, as compared with tubers from the KPN plants.

Calcium deficiency

Foliar symptoms similar to those of calcium deficiency reported on other plants were observed at three centres, on acid sandy soils with *p*H values below 5. These centres were located in Northumberland (millstone grit), Dorset (Bagshot sands) and Essex (drift gravel). Each of the soils had a low phosphate status as indicated by a chemical test for phosphate solubility and by their high positive responses to phosphate applications. These data

combined with low *p*H values were also consistent with strong phosphate fixation and low calcium status. The symptoms appeared to be less acute on the plots receiving phosphates. The characteristic changes in the plants resulting from calcium deficiency were (*a*) the foliage is a sickly dull yellowish green; (*b*) the occurrence on the leaflets, when the plants are moderately advanced in growth by the end of July, of small irregular areas of blackish brown decayed tissue (necrosis): these patches are found at the apex, along the margins and on the lamina of the leaflet; (*c*) the leaflets are thin and brittle, while the margins tend to curl over the upper surface, forming a kind of leaf-roll; (*d*) the blotching is succeeded by considerable shedding of the affected leaflets.

One of the reasons for selecting a large proportion of poor acid sandy soils for the field experiments was the desire to secure soil conditions similar to those in which effects of magnesia applications have been obtained in the United States. At no centre, however, and in neither year was any appreciable difference in growth or colour of the foliage observed between magnesia and no magnesia plots. At a few centres plants treated with magnesia appeared to develop a slightly darker foliage than those not treated with magnesia, but the difference was too small to merit emphasis. The general correctness of the observations was confirmed by analyses of the yield data, which showed only one significant positive response to magnesia in each year, as compared with 18–19 significant positive responses to phosphates and the potash respectively. Moreover, the possibility that one result in twenty, judged significant on the standard 19 : 1 may be an odd chance, cannot be ignored.

Summary

The data are based upon observations derived chiefly from twenty-four replicated manurial trials made on the potato crop in 1937 and from twenty-five further trials of a different design in 1938. They deal with the manurial and other factors that induce deficiency symptoms relating to potash, phosphates and calcium respectively on the potato plant. Leaf scorch and the other potash-deficiency symptoms on the aerial part of the plant are normally induced by NP and not by nitrogen treatment. The presence of leaf scorch on the nitrogen plots in a number of trials has been satisfactorily correlated with a high level of available phosphates in the soil, as indicated both by a chemical test for phosphate solubility and the degrees of response to phosphate applications. An increase in the level of nitrogen in the NP treatment results in the intensification of potash-deficiency symptoms. A certain interaction between nitrogen and phosphates is shown to be the primary factor in inducing potash-deficiency symptoms on the part of the plant above ground. The blackening of cooked tubers which has hitherto been assumed to indicate potash deficiency has been found to result from a combination of high nitrogen with low potash in the soil. Phosphate deficiency is induced by nitrogen and NK treatments, but more strongly by the latter. Phosphate-deficiency symptoms and not potash-deficiency ones become evident on the nitrogen plants under conditions of low phosphates and low potash in the soil. Calcium-deficiency symptoms appeared at three centres on poor sandy soils with *p*H values ranging between 4·5 and 5. Observations failed to detect signs of magnesia deficiency on the plants at any centre and in either season. The results of this observation were borne out by the absence of significant yield responses to magnesia with one exception in each season.

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THE 'BLOTCHES' ON LEAVES OF ARRAN PILOT POTATOES

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(With Plates 8 and 9)

The potato variety Arran Pilot was raised by Mr D. Mackelvie from the cross May Queen × Pepo, and is one of the most widely grown earlies. After midsummer the upper leaves usually develop characteristic grey-green blisters or blotches which may occur on any part of the leaflets, but are often restricted to the bases (Pl. 8, fig. 1). The intensity of the mottling varies from season to season and from district to district, usually being more intense in the west and north. Superficially the effect resembles those due to virus infection, and it sometimes causes difficulty in the inspection of field stocks for health certification. Attempts to reproduce the condition by grafting or inoculation from Arran Pilot to other varieties have all failed, so that it seems unlikely that it is a result of infection. Most probably it is due to some genetic factor, as similar blotches have been seen in some other of Mr Mackelvie's seedlings of Arran Pilot parentage.* Apart from a statement that the blotch is an air blister (Dep. of Agric. for Scotland, 1940), there appears to be no information on the internal changes of the affected leaflet. This paper describes their histology and development.

Material and methods

Two sets of plants grown in the garden of the Ministry of Agriculture's Plant Pathological Laboratory, Harpenden, supplied most of the material examined. One set was raised from tubers selected from Irish seed twice grown in this garden. A second set from Scottish seed was planted in a more sunny position and was more conspicuously blotched. Blotches appeared on both sets of plants about mid-July 1941.

The first collection was made in bright sunshine about noon on 28 July. Portions of the topmost blotched leaf and of several leaves above it were fixed; also the stem apex surrounded by leaf primordia. Further collections of blotched leaflets were made on 19 Aug. and 5 Sept., when the blotched parts of the older leaves had become opaque while the rest of the leaf remained uniformly translucent (Pl. 8, fig. 2). On 5 Aug. leaflets from some virus-free stocks grown on Dartmoor were fixed by Mr F. C. Bawden. Before fixation the leaflets were cut into strips of about 8 × 3 mm. and were dropped into Carnoy's fluid where they were left for not more than 1 min. They were then transferred to freshly prepared Zenker's solution and the air was exhausted. After washing, dehydration and clearing, they were embedded in paraffin wax and sectioned at 6, 8 or 10 μ according to the stage of development of the blotch. Safranin and light green or Heidenhain's iron alum haematoxylin, with or without a counterstain, were the most successful staining methods.

Each plant from which material was taken was tested for the presence of virus by inoculating its sap to tobacco. Only one plant proved to be infected and material from this was discarded. The results obtained were independent of the source of the material.

Description

Sections through blotched areas showed that the structure of the leaflet was much modified and that considerable proliferation of the tissues had occurred: e.g. above the assimilatory tissue were several layers of small cells instead of the usual single-layered epidermis. How these changes occur was found by studying young leaves before any external changes were

* *Note added to proof* 8 *Oct.* 1942. The histology of the leaves of two of these seedlings has been found to be exactly similar to that of leaves of variety Arran Pilot.

visible. The growing points and youngest leaves were normal, but in the leaves immediately above those showing the first signs of blotching histological abnormalities were seen.

The internal structure of the younger leaves and of the normal green parts of the blotched leaves is similar to that of other potato varieties examined. There is a single layer of closely packed elongated palisade cells containing numerous chloroplasts. Below this are about four layers of cells of the spongy parenchyma. In a very young leaflet these are rectangular but they soon become irregularly shaped, and in the mature leaflet are separated over most of their surface by large air spaces. These cells also contain many green plastids. The whole leaflet is surrounded by a single-layered epidermis which may contain a few faintly coloured plastids. Stomata occur more frequently in the lower than in the upper epidermis. Short, stiff hairs, which taper to a point curving over towards the apex of the leaflet, occur more frequently on the upper surface. They consist of about three cells, the largest and basal one being in direct connexion with about four small epidermal cells. The outer walls of the epidermis and the hairs are thickly cuticularized.

The first change from the normal is the necrosis of an area of epidermal cells (Pl. 8, figs. 3–7). Their walls and contents become faintly brown, then they collapse and show a strong affinity for basic dyes. Necrosis occurs equally frequently in lower and upper epidermises. In any particular leaf area, both epidermises may be necrotic (Pl. 9, fig. 2), or only one (Pl. 9, fig. 1). When the upper epidermis becomes necrotic, the palisade cells nearly always proliferate. Except in the region of a vein, proliferation occurs on the lower side only when the leaf is immature with the air spaces of the spongy parenchyma undeveloped and when the tissue on the upper side of the same leaf is also proliferating.

If, as occasionally happens, a few cells in the semicircle of epidermis below one of the larger veins become necrotic, there is a slight amount of cell division whether or not the cells above it on the upper surface are affected (Pl. 8, fig. 6). The parenchymatous cells in this region are closely packed, and as very few cells are affected the result is not noticeable externally. The guard cells of the stomata (Pl. 8, fig. 7) and those epidermal cells in direct connexion with the hair bases (Pl. 8, fig. 4) remain alive for a considerable time after the other epidermal cells have collapsed. As further development seems never to occur unless the tissue immediately outside is necrotic, often a small piece of normal tissue is found below a hair base and is entirely surrounded by a mass of proliferating tissue (Pl. 8, fig. 4). Ultimately the hair and its base die and then the cells below rapidly overtake in development the surrounding cells.

As the upper epidermis collapses, there may be a very slight increase in the length of the palisade cells immediately below (Pl. 8, fig. 3). The nucleus of a resting palisade cell usually lies against the long wall about half-way down its length. It now passes into the centre of the cell vacuole and becomes active. A normal mitosis takes place, the axis of the spindle lying parallel to the length of the cell (Pl. 8, fig. 3). A cell plate forms across the centre of the spindle and the cell is divided into two approximately equal daughter cells which are about twice as long as they are wide. The lower one passes into a resting condition, but the upper again divides (Pl. 8, figs. 4, 5). This process is repeated several times, only that cell which is nearest the exterior dividing, until a tier of cells vertically above each other is formed. After the first increase in length of the palisade cell, there seems to be no further growth in size so that the newly formed cells are progressively smaller. There is also no increase in the total number of plastids. The first formed of the new cells contain a few,

but the last formed are usually entirely devoid of plastids (Pl. 9, fig. 2). In the fixed material examined the number of layers of new cells did not exceed six, but in some material taken from the Rothamsted allotments and examined fresh twice that number was found. When proliferation does occur on the ventral side of the leaflet, it is almost exactly similar to the occurrence on the dorsal side. All the new tissue is derived from the outermost cells of the spongy parenchymatous tissue or from the outermost of the rounded cells below the vein (Pl. 8, fig. 6). As on the upper side, it is only the outermost daughter cells which divide again. When the outermost spongy parenchymatous cells give rise to extra tissue, the inner ones develop and separate from each other in a normal manner (Pl. 9, fig. 2).

When several additional layers of cells have been formed the affected part of the leaflet appears greyish green. This is due to the partial masking of the green by the discoloured epidermis and by the presence of a mass of colourless cells outside those containing chloroplasts (Pl. 9, figs. 1–3). At this time a section through a blotch shows a leaflet to be bounded on the upper side by a necrotic epidermis which may become cracked or torn away in places. Below it are several layers of thin-walled cells. The upper ones are elongated parallel to the leaf surface, and apart from the nuclei are almost devoid of visible contents. The lower are more nearly square in section and contain a few chloroplasts. In fixed material the walls of these cells give only cellulose reactions. Below this is a layer of cells similar to the original palisade but little more than half their length: these were derived from the first division of the palisade cells. Below these are several layers of the irregularly shaped cells of the spongy parenchyma. On the lower surface may be a normal epidermis (Pl. 9, fig. 1), a necrotic epidermis (Pl. 9, fig. 3), or several layers of small thin-walled cells bounded by a necrotic epidermis (Pl. 9, fig. 2).

As the increase in cell size is slight, the blotch is seldom thicker than the rest of the leaf. It is often deformed, however, the upper surface being depressed and the lower bulging slightly (Pl. 9, figs. 1, 3). Such deformation is usual when proliferation is on the upper side only.

Soon after the blotch becomes visible externally the tissues within it begin to degenerate. The last formed of the cells, which are small and almost devoid of visible contents, remain unchanged, but the contents of the larger cells shrink from the walls and large air spaces form between the cells (Pl. 9, figs. 2, 3). After 3–4 weeks the tissue seems to be completely dried out. If examined against a light, the opacity of the blotched area compared with the translucence of the rest of the leaf suggests that the former contains much air (Pl. 8, fig. 2).

Only once was the tissue within a necrotic epidermis found to collapse without a previous proliferation. The contents of the palisade cells disappeared but the spongy tissue was unaffected (Pl. 9, fig. 4). Occasionally, when no proliferation follows necrosis of the lower epidermis, necrotic material may extend inwards between the cells of the spongy parenchyma (Pl. 9, fig. 3).

Discussion

Although there are a number of effects in other plants that resemble the Arran Pilot blotch in one or other of its features, none previously described is quite comparable. The chloroses caused by virus infection, although externally similar, are strikingly different internally, for these are due to inhibition of the development or to the destruction of chloroplasts or their pigments. The silvering of leaves of fruit trees infected with *Stereum purpureum* produces over a wide area an external appearance similar to that of the blotched areas, but there the

internal changes again differ, for the silvery appearance is due to separation of the mesophyll cells from each other and from the epidermis to form air spaces (Brooks, 1928). The proliferated tissue of the blotched areas is also reminiscent of the epidermis or hypoderm of several cells thickness characteristic of some plant species, usually xerophytes. Such cells, however, are presumably differentiated in the leaf primordium, whereas in Arran Pilot they are formed by adult cells becoming meristematic.

Perhaps the closest parallels are found in some plants infected with viruses causing deformities rather than mosaic. In narcissus with stripe (Caldwell & James, 1938) groups of a few palisade cells elongate and push their way between the epidermal cells so that ridges are formed on the leaf surface. The palisade cells may divide but not apparently to form as many layers as in the Arran Pilot blotch. A further comparison might be made with 'enations'. These outgrowths from the lower leaf surface may be of genetic origin or a symptom of virus infection. In the former case (e.g. *Nicotiana tabacum* var. *deformis*), groups of cells below and slightly to the side of a vein remain meristematic after the rest of the leaf is fully differentiated and develop into flaps of tissue which assume the exact structure of the leaf lamina. In cucumber with ring-spot virus groups of cells, similarly placed near a vein, which had ceased to divide assume again their meristematic property on stimulation from the virus.

The development of the meristematic cells from those which normally have ceased to divide is a well-known reaction of plants to wounding. In previously described reactions, however, the characteristic feature has been the formation of a definite cambium layer, whereas in Arran Pilot it is the outer daughter cell which divides. In spite of this difference, it is simplest to regard the proliferated tissue as a wound reaction, the necrosis of the epidermis providing the stimulus. Nuclear division in adult cells has several times been found to follow necrosis caused by virus infection, although in such conditions there was no cell division. Although this may explain the proliferation of the tissues, no adequate explanation can be offered for the death in small areas of the epidermis of leaves formed late in the life of the plant. That it is genetic seems certain, and its irregular distribution suggests that it may resemble chimaeras.

Summary

About flowering time a greyish green blotch appears on some of the leaves of Arran Pilot potato plants. It is due to necrosis of the epidermis, followed by cell division in the palisade tissue resulting in the formation of several layers of small, thin-walled colourless cells. This proliferation may occur on the top only or on both sides of the leaf. The new tissue partially masks the green colour of the plastids in the cells nearer the centre of the leaf. After a few weeks the central tissue dies. The blotching, which is almost certainly of genetic origin, is discussed in comparison with other plant effects which resemble it in one or other of its characters.

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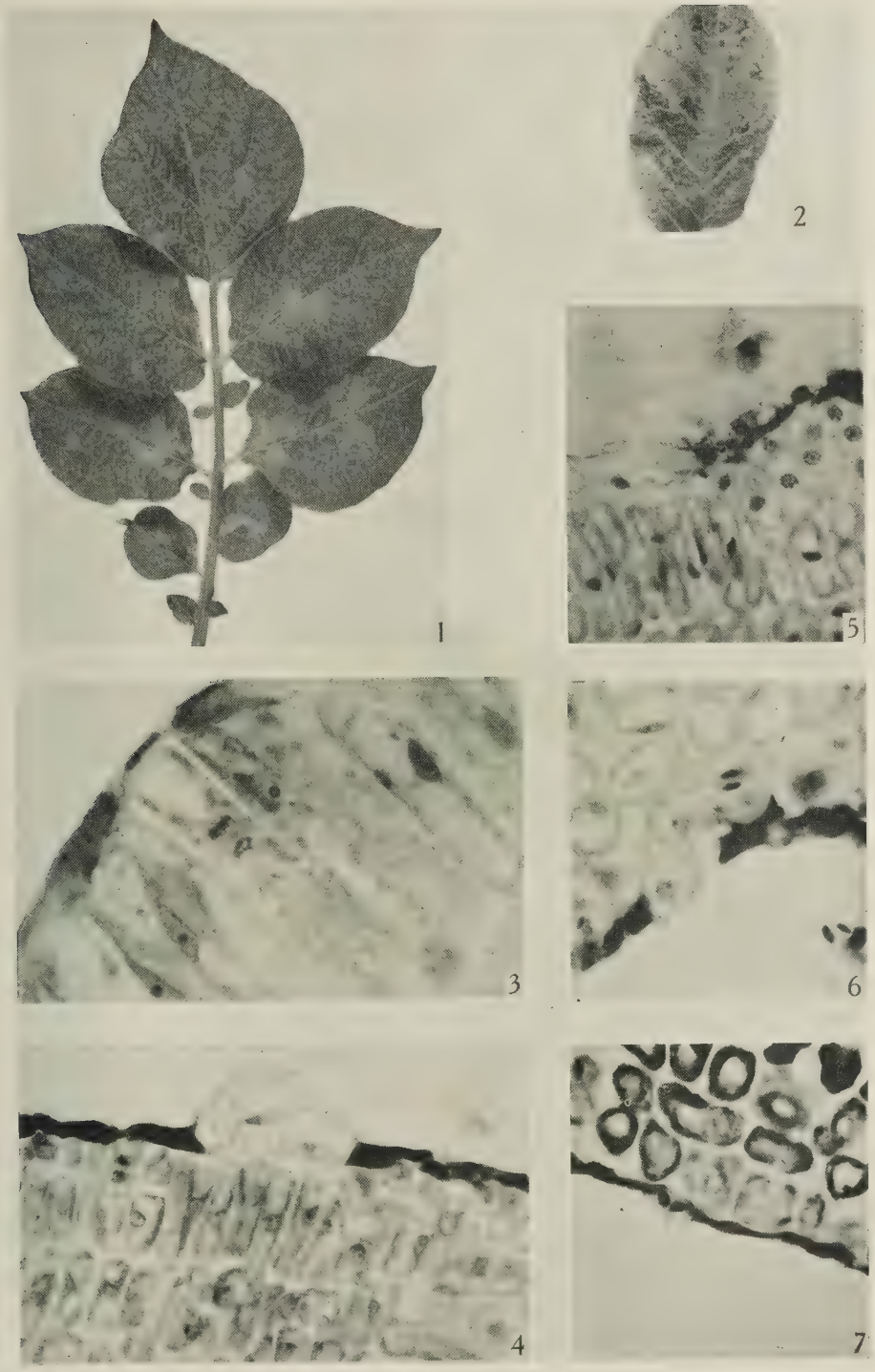
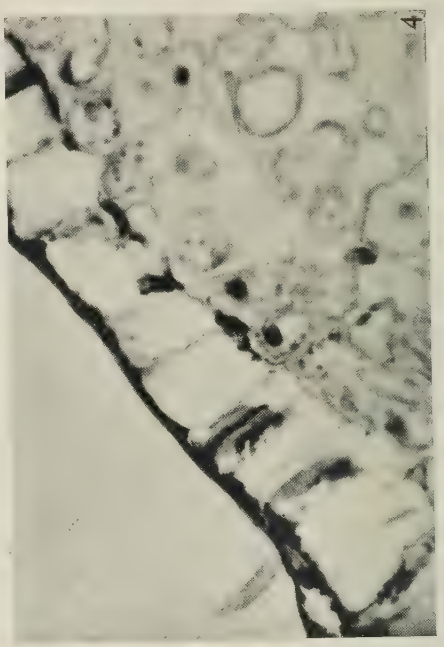
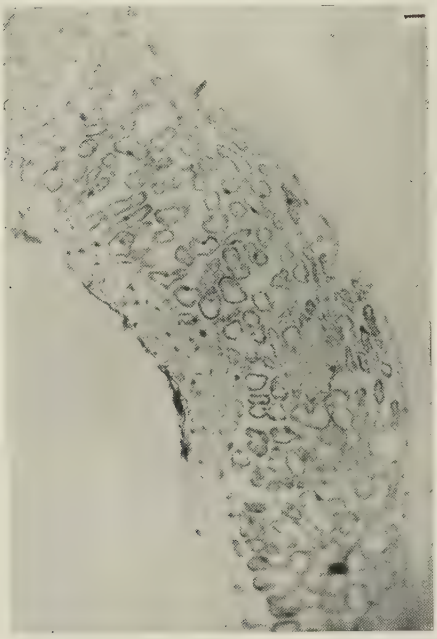
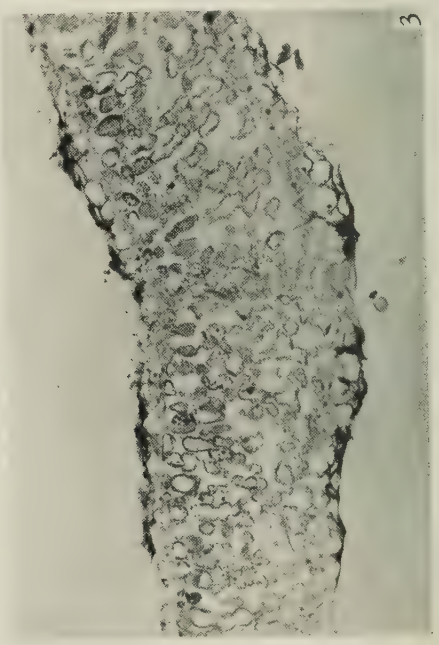


SHEFFIELD—The 'blotches' on leaves of Arran Pilot potatoes







SHEFFIELD—The 'blotches' on leaves of Arran Pilot potatoes

EXPLANATION OF PLATES 8 AND 9

The photomicrographs all show portions of transverse sections of leaflets. They were taken with a Leitz 'Makam' camera, the source of illumination being a mercury vapour lamp screened by a Wratten colour filter, no. 62. After the description of each figure is given, in brackets, the magnification and the stain used. (H.H. = Heidenhain's iron-alum haematoxylin; S.G. = safranin and light green.)

Plate 8

Fig. 1. A compound leaf of Arran Pilot potato photographed by reflected light shows greyish green blotches on some of the leaflets.

Fig. 2. About a month after their first appearance, the blotches become opaque to transmitted light. This leaflet was photographed in front of an illuminated screen.

Fig. 3. Some of the upper epidermal cells have become necrotic and collapsed. The palisade cells directly below have elongated slightly. The nucleus of one of them has reached the telophase of mitosis and a cell plate is forming across the centre of the spindle. (×900, H.H.)

Fig. 4. The cells of the upper epidermis are, except for a hair base, necrotic. The palisade cells directly below the hair base are normal; most of the others have divided once. In one of the upper daughter cells, the nucleus is in the telophase of a further mitosis. (×450, H.H.)

Fig. 5. Cell division has taken place below a necrotic part of the epidermis. The nuclei of these cells look like those of meristematic tissue. (×450, S.G.)

Fig. 6. Necrosis of the lower epidermis slightly to the side of a vein is followed by division of the adjoining parenchymatous cells. (×450, S.G.)

Fig. 7. The lower epidermis is necrotic but the guard cells of the stomate are still normal. (×450, S.G.) (This photograph was overprinted to show the walls of the guard cells: no significance should be attached to the darkening of the contents of the mesophyll cells.)

Plate 9

Fig. 1. Between the necrotic upper epidermis and the assimilatory tissue are several layers of small, thin-walled, almost colourless cells. The lower part of the leaf is normal. The leaf surface is concave above and bulges below. (×140, H.H.)

Fig. 2. On both upper and lower sides of the leaf are found a necrotic epidermis and several layers of proliferated tissue. The inner layers of the new cells contain a few chloroplasts. (×140, S.G.)

Fig. 3. Necrosis has occurred on both sides of the leaf but the tissue has proliferated on the upper side only. On the lower side necrotic material is seen between the cells. (×140, S.G.)

Fig. 4. An unusual effect seen in one leaf only. After the necrosis of the upper epidermis, the palisade cells lost their contents and died, while the lower tissues remained unaffected. (×450, H.H.)

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VERTICILLIUM WILT OF THE HOP (*HUMULUS LUPULUS*)*

By W. G. KEYWORTH, *East Malling Research Station, Kent*

(With Plate 10 and 2 Text-figures)

Verticillium wilt of hops was first recorded by Harris (1927) at Penshurst, Kent, in vars. Fuggle and Tolhurst. From 1930 onwards new outbreaks were reported in Kent, Sussex and Herefordshire, and by 1937 about twelve had been identified.

Verticillium albo-atrum Reinke & Berth. was isolated readily from the wood of diseased bines and from the roots and underground stem system, but no conidiophores were observed on the aerial parts of plants under field conditions. Young hop plants inoculated with the pathogen developed all the characteristic symptoms of wilt (Harris, 1936; Harris & Furneaux, 1938). From a study of the outbreak at Penshurst, Harris (1936) concluded that *V. albo-atrum* was present in most of the plants in an affected field whether the bines wilt before hop picking or not, and that the intensity of attack was related primarily to fluctuations in the seasonal and soil conditions under which a particular plant was growing, the intensity of wilt being greatest in wet seasons and in wet parts of the hopfield. As a possible method of control, growers were advised to improve the drainage system in affected fields and not to plant hops on ground which had a high summer water-table caused by springs, unless the water from these could be piped away.

By 1937 the disease was very severe in a number of Kentish hopfields and the writer was appointed to continue the investigations of the disease on an intensive scale. This paper deals with work begun in July 1938; preliminary observations made in 1938–9 have been described (Keyworth, 1939*a*, *b*).

The hop plant and its cultivation

The hop has a perennial rootstock which bears annual stems (bines). In March the stock is trimmed, and in April and May many young bines grow from buds on it; these are thinned to six or eight which grow up strings to wires stretched about 12 ft. above the hopfield. Flowers form on the lateral branches and the hops ripen and are picked in September. The bines then die back, leaving thickened basal portions which can be used as cuttings. Hops are planted symmetrically in various ways. The system most common in Kent is the *square plant* in which the hops are planted at the corners of squares of side 6–7 ft., with alleys between the plants at right angles to each other, so that the ground can be cultivated in two directions. Another system is the *Worcester plant* in which the hops are planted 3 ft. apart in rows which are 7 ft. apart, and the alleys between the hops are cultivated in one direction only. Other systems of planting differ slightly from these, but all fall into one of two categories, those in which the ground can be cultivated in two directions at right angles and those in which it can be cultivated in one direction only. These symmetrical systems of planting make it possible to map hopfields to scale indicating the exact positions of diseased and normal plants: the directions of spread of the disease may bear a definite relation to the system of planting.

Symptoms of the disease

Affected leaves develop yellow patches which usually enlarge until the whole leaf is yellow. Then irregular necrotic black areas occur between the main veins, forming a characteristic yellow and black pattern. The leaves rarely wilt but their edges become desiccated and curl slightly upward, and eventually the leaves become completely withered. In this condition

* Part of a Thesis approved for the Degree of Doctor of Philosophy in the University of London.

they are very easily blown or knocked off, thus differing from leaves on bines which have died from mechanical injury. The lower leaves show symptoms first, and progressively higher leaves become affected throughout the season. The leaf symptoms are not reliable for accurate diagnosis as the leaves of hops may turn yellow and die for various other reasons. Another symptom is the browning of the wood within affected bines. Typically, the whole of the wood cylinder is evenly light brown, but this coloration may be limited to the centre or to a sector of the wood cylinder. Brown wood within the bine is a constant symptom and, in conjunction with the symptoms shown by the leaves, enables a reliable field diagnosis of the disease to be made. The third symptom, more common in some fields than in others, and often absent even in badly affected plants, is the swelling of affected bines for 4–5 ft. upwards from the base. It is not a reliable diagnostic feature, since its absence does not indicate absence of disease and since healthy bines which are rather rank in growth often become swollen. After the hops are picked it is still possible to distinguish some of the affected bines by the presence on them of six black streaks, which run along the ridges over the primary vascular bundles of the bine.

Of the symptoms described, one only, the browning of the wood, is constant, the other symptoms being of value only when used in conjunction with the brown wood.

The causal organisms

Until 1938 the fungus isolated from diseased hop plants showing the symptoms described was invariably *V. albo-atrum*. In August 1938, however, *V. Dahliae* Kleb. was isolated from fourteen wilted plants of one experimental variety in the hop garden at the East Malling Research Station. *V. Dahliae* was later isolated from hop plants growing at the South-Eastern Agricultural College, Wye, and from plants in a commercial hopfield near Maidstone. Apart from those three outbreaks isolations from affected bines have always yielded *V. albo-atrum*.

Rudolph (1931) and others have doubted whether *V. Dahliae* is entitled to specific rank. In this study it has been found convenient for the following reasons to refer the two forms of isolates from hop to distinct species. Never have they both been isolated from the same plant or from affected plants in the same outbreak, and the main cultural difference between them, the production of different forms of resting mycelium, has remained constant. *V. albo-atrum* in culture on 1% prune agar produces a filamentous resting mycelium of dark, oblong or rounded cells which multiply by transverse division. These filaments may become curled and twisted into a mass of dark hyphae resembling a microsclerotium. *V. Dahliae* produces large numbers of microsclerotia composed of swollen cells most of which are almost spherical and often multiply by divisions parallel to the longitudinal axis of the hypha. These torulose cells become very dark and finally opaque. The cultures on prune agar of *V. Dahliae* obtained from hops produce fewer spores than comparable cultures of *V. albo-atrum*. A further point of difference, previously undescribed, is that whereas the microsclerotia of *V. Dahliae* are produced early in the growth of the culture and appear to be formed readily during the normal growth of the mycelium, the aggregates of dark mycelium formed by *V. albo-atrum* are not produced until the culture is old and when presumably 'staling' has occurred. Cultures of both *V. albo-atrum* and *V. Dahliae* rapidly lose their power of forming either dark mycelium or microsclerotia, and after being kept for 12 months in culture on prune agar many subcultures remained colourless. Neither of the two cultural forms has been observed to change to the other.

Host-parasite relationship

Many observations suggest that *V. albo-atrum* attacks hops from the soil through their roots. The distribution of the parasite within one hop plant was investigated by incubating portions of tissue taken from various parts of the plant and observing the portions after

3 days for the presence of *Verticillium* conidiophores. Of the eight roots examined, six contained the parasite, the greatest distance from the rootstock at which it was isolated being 32 in. One wilted and one unwilted bine were examined and successful isolations obtained from the entire length of the wilted bine (9 ft.), but not higher than 4 ft. from the base of the unwilted bine.

In August 1938, verticillate conidiophores and conidia were found on dead diseased leaves, and cultures made from the conidia were indistinguishable from those of *V. albo-atrum* obtained by direct isolation from the wood of diseased bines. The conidiophores formed a thin, silvery grey coating along the petiole and veins of the leaves, being more abundant on the lower than on the upper surface. They occurred in greatest quantity in small depressions on the leaf or in the fork between two veins, but were also scattered thinly all over the lamina. Spores were also found plentifully on diseased bines and leaves lying on the ground after hop-picking.

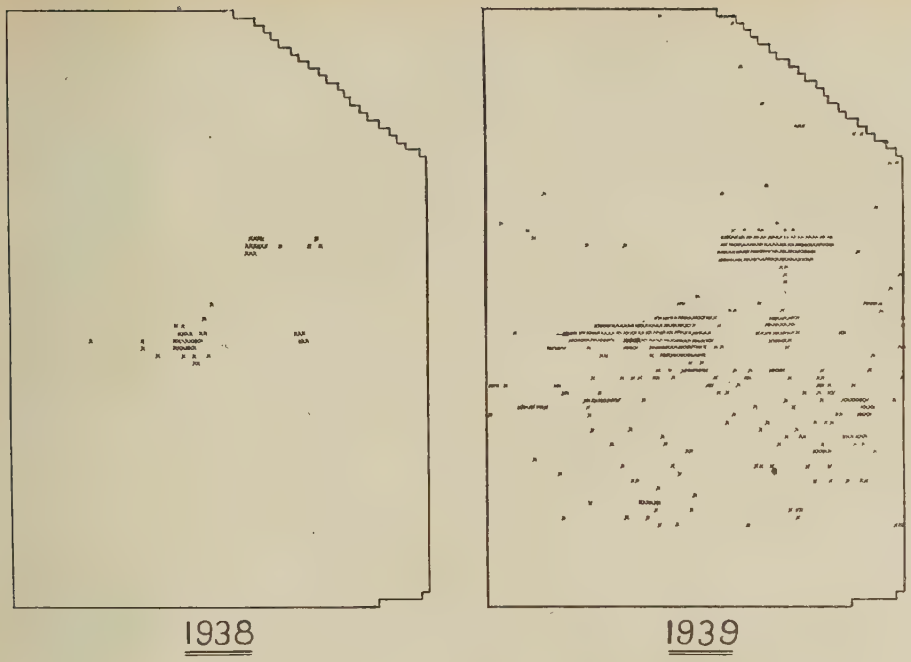
Isolations from other diseased plants showed the fungus to be present in all parts of badly diseased bines except the bracts and bracteoles of the hop cones. This invasion of the moribund plant by the fungus is a notable feature of this disease, showing that in its attack the pathogen is capable of much more than merely plugging the vessels or producing toxins. Its attack also ensures the production of great quantities of infective material (see p. 350).

The spread of the disease

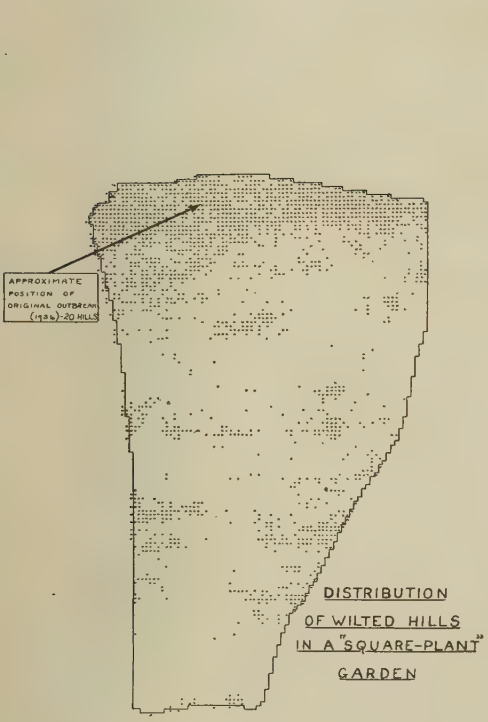
In 1938, twenty outbreaks of this disease were known in Kent and one in Herefordshire. By 1941 the number was sixty-eight in Kent and three in Herefordshire; twenty-four of these were apparently new and twenty-six were two or more years old but not previously noted; twelve outbreaks were very serious and caused the abandonment of many acres of ground for hop growing. An outbreak on one farm of 230 acres led to the loss of 25 acres of hop land, and in the past 3 years resulted in the annual loss of 5000–6000 hop plants. A study of possible means of spread of the fungus was made on certain farms with serious outbreaks of disease. The conclusions of Harris (1936) that there was no true spread of disease, any increase in the number of wilted plants being due to fluctuations in soil-water conditions (see fluctuating type, p. 351), did not appear applicable to some of the later and more severe outbreaks where observations suggested that the disease was certainly spreading, probably by the dissemination of fungal inoculum. On one large farm the spread of disease was especially rapid. Text-fig. 1 illustrates the extent of the disease in a field in the years 1938 and 1939. The disease was first noticed on this farm in 1934 when a group of about twenty wilted plants was seen in one field (see Text-fig. 2*a*). In 1938 there were 2000 diseased plants in this field, and in 1939 the number of affected fields on this farm was twenty-six out of a total of thirty-one.

During 1938, maps were made of the positions of wilted plants in certain hop fields, some with the 'square-plant' and others with the 'Worcester-plant' systems. Text-fig. 2*a* illustrates a field planted on the 'square-plant' system and cultivated in two directions. Here the disease first appeared in the position indicated, whence it seems to have spread both to adjacent plants and to plants at some distance from the original patch, which then became the foci for further spread of disease which was fairly even over the whole field.

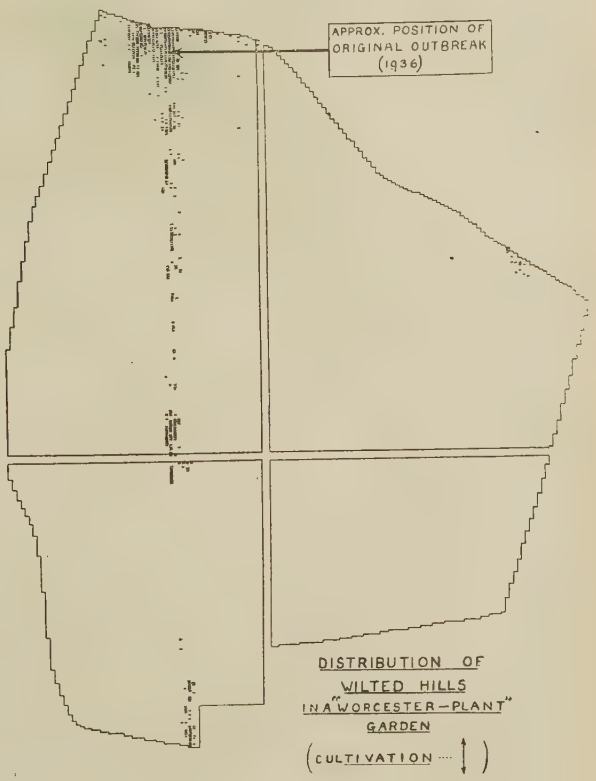
Text-fig. 2*b* illustrates a 'Worcester-plant' field on the same farm cultivated only in the



Text-fig. 1. Increase of disease in one year in one field.



Text-fig. 2*a*.



Text-fig. 2*b*.

direction indicated. The spread of disease appears to have been mainly in that one direction, the disease patches being in line and each patch being elongated. Comparison of this and other maps with those made of 'square-plant' fields suggested that disease inoculum was being transported by the cultivation operations. Pl. 10, fig. 3, shows a cultivator which has travelled 50 yd. along a hop alley early in the growing season and has gathered a great quantity of bines and leaves. If disease can be spread within one field by the cultivators it seems probable that it is spread from one field to another by the same means.

To ascertain whether diseased plant material could infect healthy plants the following experiment was made.

Soil infection experiment

In this experiment made in 1939, twenty-five plots, each 2 yd. square, were separated by paths 1 yd. wide. There were five treatments, each replicated five times and grouped into five randomized blocks, consisting of the application to the soil of the following:

(1) Two bushels of soil from around diseased plants.
(2) One half-bushel of soil from around diseased plants.
(3) One bushel of chopped diseased bines collected in September of the previous year.
(4) One half-bushel of dead leaves from diseased bines.
(5) Control—no treatment.

The plots were dug over lightly to mix the diseased material with the soil. On 24 March, 4 days after the plots had been treated, fifteen bedded Fuggle sets were planted in each plot.

Disease symptoms were first seen on 2 June 1939, 10 weeks after the sets were planted, when some leaves on a few of the plants on plots which had received diseased bine began to turn yellow. From this date the symptoms became more and more apparent on certain plots. Table 1 summarizes the records made on 3 July. The addition of diseased bine or leaves to the soil induced the wilting of a large number of plants, whereas the addition of contaminated soil induced few such cases. By the end of September most of the plants on the plots treated with bine or leaves were dead. With the exception of one diseased plant all the plants on the control plots remained healthy (Pl. 10, figs. 5, 6).

Table 1. *Wilt incidence on plots treated with infected soil and plant debris*

Treatments	No. of plants wilted in each plot of 15 plants					Totals
	Block 1	Block 2	Block 3	Block 4	Block 5	
1. Soil 2 bushels	2	0	0	3	1	6
2. Soil ½ bushel	0	0	2	0	2	4
3. Bine	15	15	15	14	15	74
4. Leaves	11	14	13	13	12	63
5. Control	0	0	1	0	0	1

This experiment shows that the application to the soil of contaminated material (especially bines and leaves from diseased plants) can induce disease symptoms in hops planted in that soil and, therefore, that such debris plays an important role in the transmission of disease in the field.

Many farm operations may cause the spread of disease. When plants are grubbed, the soil removed is often thrown in the alleys where farm machines may carry it about. Tractors, especially those with caterpillar tracks, often move soil several feet but ploughs do not transport soil to any extent. The cuttings removed when the hops are trimmed may carry the disease as also may the young bines and leaves which are later removed and thrown into the alleys. Later in the season the dead leaves are easily detached from the wilted bines and on falling to the ground are carried about by the cultivators: this is thought to be the most important means by which the disease is spread. There is also much movement of diseased material during hop picking. It was clearly shown on one farm that manuring the soil with compost

containing infected bines greatly increased the number of wilted hills. In the summer of 1937 the grower had noticed about fifty wilted plants scattered in small patches throughout an 11-acre field. The bines from this field were collected in the autumn and put in a compost heap, and the following spring this compost was spread over most of the field, the only untreated portion being about thirty rows at one end. In the summer of 1938, 650 wilted plants were found scattered over the whole of the treated portion of the field, while the part which did not receive any hop bines was free from wilt.

Leaf fragments may be blown for many yards, and a strong breeze might possibly blow them from one farm to another. Affected farms occur in groups, and many farms in which recent outbreaks of disease have occurred are very near to farms with outbreaks of longer standing. On one farm a severe outbreak occurred in one field and was followed in succeeding years by outbreaks in the four adjoining fields all of which were separated by ditches, so that there was no direct cultivation from one to the other.

The fungus is also disseminated in diseased cuttings and bedded sets, such cuttings and sets having been shown by experiment to give rise to infected plants.

Relation to other hosts

A comprehensive list of hosts for both *V. albo-atrum* and *V. Dahliae* is given by Rudolph (1931). Of these plants only two, the potato and the raspberry, have been found to be associated with hops attacked by *V. albo-atrum* or *V. Dahliae*. Isolates of *V. albo-atrum*, indistinguishable in cultural characteristics from that attacking hops, have been obtained from wilted potatoes, and potato plants have been infected by inoculating them with a hop isolate of *V. albo-atrum*.* Inoculations from potato to hop have not yet been made. In a field trial made in 1939 on ½ acre of ground on which wilted hops had grown, potatoes planted in this area contracted wilt and *V. albo-atrum* could be isolated readily from them. On one farm wilted hops were found near to raspberries affected with *Verticillium* wilt. *V. Dahliae* was isolated from both host plants, and it is probable there was some connexion between the outbreaks in the two crops.

The influence of the environment on the disease

In farm surveys made during 1938–41 attacks of wilt were found which varied widely from each other both in symptom expression and in severity, as measured by the annual number of wilted plants. Two types of outbreak have been recognized: 'fluctuating' and 'progressive'. Many outbreaks, however, could not be classified, either because they were of recent origin or because insufficient data were available on the annual severity of the disease. Fifteen of the established outbreaks seen to date fluctuate in intensity from one season to another, the affected plants being usually scattered irregularly about the field, either singly or in groups of two or three plants. Large groups of diseased plants are usually first seen in August, and in some seasons the symptoms have been reported to increase rapidly in severity until hop picking.

The main feature distinguishing fluctuating outbreaks from those of the progressive type is the common recovery of plants which have shown wilt symptoms. Certain symptoms are also more pronounced in fluctuating outbreaks, notably the thickening of the bines, the uneven yellowing of the leaves and the limitation of brown wood to the centre only of many

* *Rep. E. Malling Res. Sta. for* 1926, I, General, p. 59.

bines. The first outbreak which Harris (1936) studied in detail was of this type, and his conclusions on the incidence of the disease were based largely on this study. It is noteworthy that this outbreak has not increased in severity since its discovery in 1924, and the affected field is still in satisfactory production.

Twelve examples of progressive outbreaks have been observed. In these there is usually first noticed a group of a few plants bearing several wilted bines. If these plants are left they often die within 1–2 years. If a wilted plant is removed and the space replanted, the young plant usually contracts wilt in the first season. The disease rapidly spreads, and in the next season affected plants are found adjacent to the disease patch and in other parts of the field, by which time the disease may have spread to other fields. Symptoms of wilt are often noticeable in June and have been seen as early as 17 May. The markings on the wilted leaves are very variable but many leaves show definite black streaks between the veins: this symptom becomes less distinct later in the season. The bines rarely become abnormally thickened, and the brown wood within them can be seen immediately below the cortex. The progressive type of outbreak is of great importance to the grower because affected hills do not recover.

From observations made by Harris & Furneaux (1938) and by the writer it appears that the variations between attacks are related in some way to local soil conditions. Harris (1936), investigating an outbreak of the fluctuating type, concluded that variations in the severity of symptoms on plants in different parts of the field were correlated with variations in soil moisture and drainage, and that disease symptoms were most evident during a wet summer.

During the study of a progressive outbreak on one farm the writer has followed up these observations, studying variations in soil moisture in fields with and without wilt outbreaks. The method described by Harris & Furneaux (1938) was used in these experiments. Iron tubes pierced with small holes were sunk in the soil and the water level in these tubes regarded as an indication of the soil-water level in the immediate vicinity. Three experiments were made as follows:

Field no. 1. This was a 2-acre field near the centre of which were five wilted plants. Tubes were inserted around this wilt patch, one tube being at the approximate centre of the wilt patch and four tubes on each of four circles at increasing radial distances from this centre. The five tubes nearest the centre of the wilt patch were regarded as being in the disease area and the others were outside it. The average of the readings of all the tubes in each circle together with the averages for each circle over the whole period are given in Table 2. The average for the tube in the centre of the wilt patch over the period was 30 in. There was little difference between the readings in any of the tubes, and it was concluded that in this field over the period of the experiment there was no relation between the wetness of the soil and the incidence of wilted plants.

Field no. 2. Near the centre of this 4-acre field was a large wilt patch of about 100 affected plants. The soil was deep and showed no signs of extensive waterlogging. Twelve tubes were placed 65 ft. apart in two rows across the wilt area and were measured in the same way and on the same dates as in field no. 1. The results showed that the soil in this field during the period of the experiment was very dry both in, and outside, the wilt area. It was therefore apparent that during the period of the experiment the incidence of wilt symptoms bore no relation to the wetness of the soil. Wilt symptoms were shown by plants growing in a soil in which the depth of the water level below the surface was never less than 49 in.

Field no. 3. No wilted plants had been observed in this 5-acre field during 1938, but the soil of certain parts was found to be waterlogged at a shallow depth. Five tubes were inserted about 70 ft. apart in a row passing through the centre of the field, and their water levels were measured in the same way and on the same dates as the other tubes. One tube consistently had the highest water level, and soil borings confirmed that the soil in this part of the field had a high water-table, but this did

not induce wilt symptoms, and it was evident that the absence of wilt in this field bore no relation to the soil-water levels during this period.

TABLE 2. *Soil-water tube measurements in an affected field*

Distance of circle from centre ft.	Distance of water surface from top of tube (in.)										Average over whole period
	14 Feb.	20 Feb.	27 Feb.	6 Mar.	20 Mar.	2 May	15 May	22 May	26 June	10 July	
6	30	31	32	27	30	15	31	35	36	38	34
12	30	32	33	21	31	15	31	35	40	41	31
24	30	32	33	30	31	16	29	32	33	35	30
48	30	32	32	29	29	11	27	31	35	38	30
96	29	31	30	26	30	12	40	33	46	40	31

The conclusion was that, on this farm during the period of these experiments, the occurrence of wilt symptoms was not primarily related to local variations in soil-moisture conditions, and that the disease could be found in hops growing in soils with widely differing water conditions.

Four very severe progressive attacks occurred on the alluvial soil of the Medway plain, many parts of which become waterlogged in winter. The mildest outbreaks of the fluctuating type occurred on deep well-drained soil lying over sandstone, but outbreaks on soil over sandstone are not invariably fluctuating, and until more information is available on the soil structure of affected fields no definite conclusions can be formed on the possible correlation of differences in the nature of outbreaks and differences in soil conditions.

In an effort to determine whether variations in outbreaks were correlated with variations in the pathogen many isolations were made from diseased hops in outbreaks of all types and their cultural characteristics compared. No tests of relative pathogenicity have been made, but on the evidence at present available it seems improbable that the differences between outbreaks are primarily related to differences between the strains of fungi present.

THE CONTROL OF THE DISEASE

The problem of control differs for the fluctuating and progressive types of outbreak. The former was studied in detail by Harris, but the present writer has mainly concentrated on the latter and more immediately serious type of outbreak. The control of soil-borne wilt diseases is not easy, and the difficulties are increased when, as in the hop, the host plant is a perennial. In addition, on account of the high capital cost of hop growing the abandonment of infected ground is a great loss to the grower. Efforts at first were chiefly directed to checking the spread of this disease throughout the hop-growing districts rather than to attempting its eradication where already firmly established, but it has latterly been possible to devote some attention to possible methods of eradicating the large outbreaks. Rudolph (1931) summarizes the main control measures which are possibly applicable to outbreaks of *Verticillium* wilt in field crops as: (1) field sanitation, (2) soil disinfection, (3) resistant varieties, (4) crop rotation.

(1) *Field sanitation.* Certain hygienic precautions were suggested by the observations detailed above (pp. 348–51) on the pathogen and on the disease in the field. These precautions provide the principal methods at present used for checking the disease and are described in detail below (p. 355).

(2) *Soil disinfection.* Sterilizing soil in the field by heat being impracticable, the only methods investigated have been the application of chemicals.

Field trial of certain chemicals as soil disinfectants. Twenty-four plots each 3 × 2 yd. were laid out on a piece of naturally infected ground and separated by paths 1 yd. wide, left untreated. The plots were first dug to a depth of about 6 in. and then the treatments shown in Table 3 were randomized over the twenty-four plots. The solutions were applied from a watering can fitted with a coarse rose. The 8 gal. of 2% formalin were applied in two equal doses on successive days and all the others in one dose. The soil at the time of treatment was moist but not very wet. Two gallons of liquid were easily absorbed, but in the larger doses some of the liquid was not absorbed and ran to the edges of the plots. The plots were lightly dug within 1–2 hr. after treatment, and where 4 gal. of liquid had been applied the soil was saturated to a depth of about 6 in. The solid copper sulphate was powdered and scattered evenly over the plots. The pentachlorethane was applied with a Vermorel soil injector at a depth of 4 in., 150 injections per plot. When the second plot was being treated the injector leaked and an overdose of about 50–100 c.c./sq. yd. was given. This chemical was used in the hope that its vapour in the soil would kill the fungus to some distance from the point of application. Ezekiel & Taubenhaus (1934) reported that the application of this and similar volatile chemicals to the soil killed the fungus *Phymatotrichum omnivorum* on cotton roots at a depth of 2 ft. below the point of application. All the plots were left for 2–3 weeks after treatment and then twenty bedded sets of the Fuggle variety were planted in each. The results as finally recorded on 8 Aug. 1939 are shown in Table 3. Sixteen plants on the plots treated with solid copper sulphate were very stunted, and all the plants on the plot which received an overdose of pentachlorethane were killed.

TABLE 3. *Field trial of chemicals as soil disinfectants against* V. albo-atrum

Chemical	Application/sq. yd.	No. of plots	No. of plants	No. diseased	% diseased
2% formalin	8 gal.	4	80	5	6
2% formalin	2 gal.	4	80	22	27
1% $CuSO_4$	4 gal.	4	80	29	36
Solid $CuSO_4$	½ lb.	2	40	5	12
Proprietary sterilant	6 gal.	2	40	26	65
Pentachlorethane	250 c.c.	1	20	8	40
Pentachlorethane	Over 250 c.c.	1	20	—	—
Control	No treatment	6	120	56	46

The application of 8 gal. of 2% formalin to the soil gave a marked reduction in the number of diseased plants and was the most satisfactory treatment, although even this large dose of formalin failed to eradicate the disease. The other treatments either did not reduce the amount of disease or killed many of the plants. Treatment of the soil with formalin is now advised as part of the measures designed to eradicate small outbreaks. It has also been used extensively on one farm in an effort to check the spread of large outbreaks and to reclaim wilt-affected areas; although its use has not resulted in any striking reduction in disease incidence, it appears, in conjunction with field sanitation, to have reduced the rate of spread of the disease. The use of formalin in the field presents great difficulties. In the winter the ground is often waterlogged, and penetration is very poor. The liquid tends to run into channels in the uneven ground and the centres of lumps of soil over 3 in. diam. remain quite dry. The carting of large quantities of liquid is difficult, but methods whereby the formalin is pumped through existing underground mains to the affected fields are now being investigated. The use of other chemicals is still under experiment, to try to discover some solid disinfectant which could be scattered on the ground and then ploughed in to check or eradicate the fungus.

(3) *Resistant varieties.* An experiment planted on the randomized block system was started in February 1939 in a commercial hopfield in which eleven commercial varieties

(Eastwell, Rodmersham, Petham and Canterbury Goldings, Mathon, Tutsham, Bramling, Early Bird, Cobb, Colgate and Fuggle) and five other varieties were tested for resistance. In 1938, 75 % of the plants in the area used for the experiment were diseased, these plants being fairly evenly distributed. All the hops in this area were removed during the winter of 1938. In 1939 disease was first noticed at the end of June, and the number of plants showing symptoms increased throughout the season. On 30 Aug. 1939, about half the plants were wilted or missing. There was no significant difference between the number of healthy plants of each variety and no differences in susceptibility to the disease appeared in the second and third season of growth. It thus appears improbable that the planting of any of these varieties in affected fields will provide an effective control of the disease.

Many new varieties raised by Prof. E. S. Salmon at the South-Eastern Agricultural College, Wye, have proved to be susceptible, but certain of these varieties have remained healthy when planted in disease areas. On one farm two other varieties have been noted which have shown no obvious disease symptoms although they have grown adjacent to a wilt area for 3 years. These varieties are being propagated and tested for disease resistance.

(4) *Crop rotation.* Since the hop is a perennial, a short-term rotation is impossible and the only form of rotation which would be practicable is the growing of an immune crop for some years on wilt-affected ground. An experiment was started in 1941 to determine whether the parasite had died out of a wilt area in which no hops had been grown for $2\frac{1}{2}$ years. An area of $\frac{3}{4}$ acre (in which, during 1938, 496 plants wilted out of a total of 835) was planted with 1653 young hops: twenty-four showed wilt symptoms during 1941. Thus the effectiveness of the soil as a source of infection was greatly reduced during the $2\frac{1}{2}$-year period without hops.*

Summary of recommended control measures

(1) *General hygienic measures.* No bine from an affected field should be used as manure for hops even though it has been put in a compost heap: all such bines should be burned in the field after hop picking. No cuttings should be taken from an affected field, but if no other source of cuttings is available they should be taken from parts of the field as far away as possible from the affected area. Hops should not be planted after either potatoes or raspberries. Such hops would contract wilt only if the potatoes or raspberries themselves were diseased, but since wilt may be present in these crops without being very noticeable they should always be regarded as suspect. Where large numbers of wilted hops have been removed the ground should not be planted with potatoes. Crops suggested for planting in such ground are cereals, root crops, and vegetables such as runner beans, cabbage, kale, sprouts, lettuce, onions, etc. The planting of soft fruits in such areas is not recommended as some of them are susceptible to *Verticillium* wilt.

(2) *Control measures applicable to the progressive type of attack.* To ensure that the cultivators and tractors do not carry contaminated soil and plant material from one field to another, they should be washed with a pressure hose after an affected field has been cultivated. Alternatively, all the clean fields should be cultivated first, then the affected ones, leaving the worst until last. All cuttings, excess bine, and stripped leaves from plants on and near disease patches should be gathered and burned. If infection is widespread the bines and leaves from the whole field should be burned.

* *Added to proof* 11 *Oct.* 1942. In 1942 there was 10 % of infected plants on the plot, showing that the fallowing period had not adequately controlled the disease.

A close watch should be kept during the growing season for signs of wilt, and as soon as a plant shows definite symptoms the bines should be cut down. Some of the leaves fall off and care must be taken that such leaves are picked up and burned together with the bines. The stock can be left in the ground until a more suitable opportunity occurs for removing it, but when this is done the soil should not be scattered but replaced in the hole made, and the place then clearly marked, so that the grower can observe the surrounding plants for symptoms of wilt. The spaces where wilted hops have been removed should not be replanted. If a large number of wilted plants occurs in one patch it is advisable to grub the whole area. Any cultivators subsequently used on this ground must be cleaned before being used among hops.

Owing to the scattering of leaves and bine by the pickers there is probably a considerable spread of disease at hop picking, and the grower should ensure that all wilted bines are removed before this time. Wherever possible all disease areas and surrounding plants (e.g. a belt of five plants wide) should be picked separately and all leaves in these areas picked up and burned.

(3) *Control measures applicable to the fluctuating type of attack.* The measures to be applied are similar to those suggested for the progressive type of attack, but as the plants may recover it is inadvisable to remove them. The grower should adopt as many of the hygienic precautions as possible, and if the disease is known to be most severe in a wet area improvement of the drainage should be attempted.

(4) *Control measures applicable to a small outbreak.* If the outbreak is the first one on the farm and involves only a few diseased plants the grower should endeavour to eradicate the disease. The control measures described for the progressive attack should be followed strictly, and in addition the following measures should be adopted: cut down and burn all the bines of diseased plants and *treat every adjacent plant similarly*. Then excavate a hole 1 yd. square and 1 yd. deep at each place where a treated plant has been and remove the soil from the hopfield. Pour 8 gal. of 2% formalin into and upon the walls of this hole and then fill with uninfected soil. Then treat the whole area where plants have been removed with 8 gal./sq. yd. of 2% formalin, retaining this with a bank if necessary (Pl. 10, fig. 2). The spaces may be replanted after 3–4 weeks, but the young plants and surrounding old plants should be observed for wilt during the next season, and the procedure should be repeated if any wilt is seen.

Summary

A study of *Verticillium* wilt of the hop (caused mainly by *V. albo-atrum* but occasionally by *V. Dahliae*) indicated that disease outbreaks varied widely in severity and persistence, some fluctuating in intensity from year to year and others becoming progressively more extensive. Early observations on outbreaks of the progressive type suggested that the disease was being spread during the cultivation processes. Experiments made from 1939 to 1941 supported this view and showed that diseased leaves and bine were important agents in such spread. The disease is also spread by the planting of infected cuttings. The disease in hops has been found on some farms to be related to the growing of either potatoes or raspberries. Experiments on soil disinfection have been started and 2% formalin has proved promising when applied at the rate of 8 gal./sq. yd. Experimental control measures have been formulated, consisting mainly of hygienic practices designed to remove sources of inoculum.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Fox Photos, Ltd.


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KEYWORTH—*Verticillium* WILT OF THE HOP (*Humulus Lupulus*)

The writer wishes to acknowledge his indebtedness to the many hop growers who have granted facilities for his experiments and in particular to the Directors of Messrs Whitbread and Co., Ltd., and to the Manager and staff of Messrs Whitbread's Beltring Hop Farm without whose co-operation many of the experiments could not have been made; to Dr D. L. G. Davies for his help in certain of the experiments; to Dr W. M. Ware and members of the South-Eastern Agricultural College, also to Mr L. Ogilvie and Mr F. H. Beard for valuable co-operation. Thanks are due to Mr R. V. Harris for facilitating access to his unpublished data on the disease, and to Dr H. Wormald for his unfailing help and advice throughout the whole of the investigation.

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EXPLANATION OF PLATE 10

Fig. 1. Part of a diseased hop-bine showing leaves with typical symptoms.
Fig. 2. The application of formalin to a small wilt area in a commercial hopfield.
Fig. 3. A cultivator which has travelled 50 yd. along a hop alley and gathered much bine.
Fig. 4. A portion of a commercial hopfield showing numerous wilted plants.
Figs. 5, 6. Soil infection experiment. Fig. 5. Control plot. Fig. 6. Plot receiving wilted bines.

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SUGAR-BEET YELLOWS VIRUS

A PRELIMINARY ACCOUNT OF EXPERIMENTS AND OBSERVATIONS ON ITS EFFECT IN THE FIELD

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(With Plates 11 and 12)

Curly top, the most serious disease of sugar beet, does not occur in Britain. Sugar-beet yellows is, however, present and is underestimated as a potential danger to sugar production. Its symptoms, principally those of chlorosis, thickening, and brittleness of the leaves, especially late in the season, have long been known in commercial crops without their significance being appreciated. In this country the disease was only identified in 1940, although the suggestion that it might be present was put forward by Petherbridge & Stirrup in 1935. There is no detailed account of symptoms in the field or of the conditions which influence the occurrence and severity of the disease, and no data on the loss of yield which it causes. Work carried out in 1940 and 1941 suggests that war-time conditions may tend to increase the economic importance of the disease.

Symptoms and occurrence in the field

In most virus diseases the symptoms appear mainly on young, actively growing leaves, the older leaves, except in long-established infections, being comparatively normal. In sugar-beet yellows the symptoms are generally most conspicuous on the older leaves, and as nutrient deficiencies often produce similar symptoms of chlorosis and necrosis, also affecting these leaves, confusion has occurred in diagnosis, and the virus disease has tended to be overlooked.

Plates 11 and 12 show photographs of leaves taken from plants suffering from yellows. The specimens were from the second experiment described in this paper. Almost identical leaves occur in many commercial fields, but experimental beets were used because they had virus-free controls growing in the same conditions, but showing none of the symptoms illustrated. There was a little flea-beetle damage on all the plants. The photographs are on the same scale, of leaves at about the same stage of development, and taken at the same time. Differences in the sizes of the leaves and in the severity of the symptoms are due to differences in the dates on which the plants were infected. Those infected in June and July (Pl. 11, figs. 1–6) show severe stunting and necrosis. August infections (Pl. 11, figs. 7, 8, and Pl. 12, figs. 1, 2) show only slight stunting and less necrosis. Those of September or later show no stunting or necrosis (Pl. 12, figs. 3, 4) as the plants were fully grown at the time of infection, and the symptoms were localized. Though the photographs indicate a fairly wide variety of symptoms, the appearance of infected plants is even more variable in the field, where conditions of growth may vary, as well as sowing and infection dates.

The symptoms in the field which are now recognized as typical of yellows are probably the result of August or early September infections in well-grown crops. They are of intermediate types, generally similar to Pl. 11, figs. 7, 8, or Pl. 12, figs. 1, 2. Later infections

are common, but appear to have little effect upon the general growth of the plants, and as the most colourful of these late symptoms occur in well-nourished and well-grown beets, they are sometimes looked upon by growers with approval as signs of the crop 'ripening off nicely'. In the experiments recorded here virus-free beets remained green until harvest.

The chlorotic areas on virus-infected leaves vary from pale, watery or greenish yellow, to rich orange, or even red in some varieties of sugar beet. Usually the yellow parts of the leaves are also thickened and brittle, and crackle when broken with the fingers. The green parts of affected leaves, and the unaffected leaves of slightly affected plants, remain normal in texture. The chlorotic areas of the leaves generally feel waxy, or, in badly affected plants which have all their leaves yellowed, dry, and the plants rustle when shaken or brushed in passing. The leaves of these plants splinter when crushed, and do not wilt easily in dry weather.

Most viruses cause a typical symptom pattern in any particular plant, but the chlorotic pattern caused by yellows varies considerably. The discoloration may start anywhere on the leaf, but in early infections it generally spreads downwards from the tip, sometimes avoiding the veins to form a sort of interveinal mottle, and sometimes including them so that the chlorotic area is unbroken. At other times the infection starts towards one margin of the leaf, and only one half of the leaf becomes affected. Leaves infected early nearly always become necrotic, the necrosis starting at the point where they first became chlorotic. The necrotic areas frequently become invaded by fungi; a species of *Alternaria* was very common on the experimental plants. The necrotic areas on the leaves illustrated in Pl. 11 were attacked by it, but none of the control plants was affected.

The necrosis follows the chlorosis down the leaf, and forms an apical or marginal necrosis. The chlorotic symptoms seem to require optimal growing conditions for development, and in dull or cold weather the necrotic symptoms may overtake the chlorotic, so that none of the typical brittle, yellow areas can be seen. If this happens when the virus has attacked only the tips and margins of the leaves, the appearance might be mistaken for potash deficiency. Frequently, after a spell of bad weather following good growing conditions, or when plant growth is checked in some other way, all the infected leaves become necrotic, and die (Pl. 11, fig. 3). If conditions for growth remain unfavourable the new leaves which develop may be green though rather stunted and leathery, or even brittle. The result is a patch of stunted plants, each with a ring of dead leaves round it, or with some of its leaves necrotic or discoloured at the tips. These atypical conditions are difficult to diagnose, and are not uncommon. They generally occur in crops which are growing badly for other reasons, and are also most likely to occur where virus infection is widespread, as poor growing conditions increase aphis reproduction (see p. 364). As symptoms are often so little typical of what one would expect from the name 'yellows', there is probably a tendency to attribute to other causes losses in yield due to virus infection.

Two types of distribution of infection are common in field crops. There may be patches of varying size, in which practically all the plants are infected; or infected plants may be 'peppered' over the field singly or in groups of two or three. The patchy type of distribution probably occurs when the initial infestation by winged aphides migrating from an infected source takes place fairly early in the season, there being only a few viruliferous individuals which cause a few scattered foci of infection in the field. If subsequent aphis multiplication is moderate, apterae wander from the originally infected plants to their neighbours,

transmitting the virus and giving rise to the typical localized patches of infected plants. When aphis infestation is early, the plants in these patches receive their infection at a comparatively early age, so that they generally develop the bright golden colour, and are conspicuous against the dark green background of the healthy beet. The size of the patches will depend upon the earliness of the infestation and on the rate of movement of the aphides. If conditions are exceptionally favourable for rapid aphis reproduction the combination of aphis and virus attack may destroy the plants in the middle of the group. In poor growing conditions the chlorosis may be masked by necrosis and the plants will merely be scorched and stunted, instead of yellowed. This patchy distribution of yellowed, scorched, or dead plants, has tended to increase the confusion of the virus disease with mineral deficiency, as similar appearances in the crop can be caused by local irregularities in the soil.

The 'peppery' distribution probably arises from later aphis infestations containing a higher proportion of viruliferous migrants, which cause a large number of scattered infections on their entry into the crop. The spread of infection from these plants is likely to be slower than from an early infestation, as the plants would be more mature: in our experience it is more difficult for aphides to establish themselves on large, well-grown plants than on small and immature ones.

Effect of yellows virus on yield

Experiments were carried out at the Rothamsted Experimental Station during 1940 and 1941 to determine the reduction in yield of roots and sugar caused by virus infection in varying conditions. *Aphis fabae* (Buct.), and *Myzus persicae* (Sulz.) were used to transmit yellows virus to experimental plots of sugar beet while control plots were kept, so far as possible, free from infection. It was necessary to use the aphis vectors as the virus is not transmissible mechanically. The experiments were laid out on land of uniform fertility, and all plots were given an adequate basal dressing of sulphate of ammonia, muriate of potash, and superphosphate, applied at the time of sowing.

Experiment I (1940). There were twelve plots, containing vars. Kleinwanzleben E and Marsters, sown on 8 Apr., 5 May and 24 May. Two covered muslin cages, 6 ft. × 6 ft. × 22 in. high, were placed on each plot, and the remaining third of the plot was left uncovered. It was not possible to make the cages completely aphis proof, because soil was continually washing away from the wooden base board, leaving spaces at ground level. It was found that caging alone had a very serious effect upon the yields of roots and sugar, and also on the loss caused by virus infection, which was much greater in Exp. II when none of the plants was shaded.

Viruliferous aphides of both species were introduced into one cage on each plot on 28 June. *Myzus persicae* did not reproduce to any extent and few specimens could be found a few days after introduction. *Aphis fabae* decreased in number in the earliest sown cages, increased slowly in those of medium sowing date, and very rapidly on the latest sown beet, producing a winged generation by 26 July, when all cages were thoroughly sprayed. There was no artificial infestation of the uncaged plots. A few naturally occurring winged aphides were found on them in mid-July, and these produced colonies of apterae which reached their highest numbers on the latest sown plots. None was found in the cages which did not receive viruliferous aphides. These observations showed that late sowing greatly increased the susceptibility of sugar beet to aphis infestation.

A few plants on the uncaged subplots were infected by virus introduced with the natural aphis infestation, but the resulting percentage of infection was small and localized, as there was little spread between subplots, these being partly screened from each other by the cages. The results are given only for the healthy plants on these subplots. In the cages which received viruliferous aphides all the plants became infected, though some of those in the earliest sown cages remained healthy in appearance for

some time, and the symptoms were, on the whole, less severe than on the later sown beet. All the plants in the control cages remained healthy except in one cage, in which a few infected plants appeared late, and were rejected at harvest.

For the present purpose it is sufficient to consider only the yields of roots and sugar, which are given in Table 1 as tons/acre, and as means for the two varieties, since the treatment effects were very similar for Kleinwanzleben E and Marsters. On the average of all sowing dates caging reduced the yield of roots and sugar nearly to one-third, and it is assumed that this effect was due to shading by the muslin. Shading slightly reduced the percentage of sugar in the roots as well as the total weight.

TABLE 1. *Yield of washed roots and of sugar, and percentage reduction of yield by shading and by infection (means of two varieties). Exp. I*, 1940

	Mean yield in tons/acre				Reduction of yield, %	
	Treatment of plants					
Sowing date	Uncaged healthy	Caged healthy	Caged infected	S.E.*	Of healthy plants, by shading	Of shaded plants, by infection
			Roots			
8 Apr.	17·44	6·68	6·33	(*a*) 0·453	62	5
5 May	16·36	5·80	4·30	(*b*) 0·406	65	26
24 May	13·86	5·34	3·51		61	34
Mean	15·89	5·94	4·71	0·234	63	21
			Sugar			
8 Apr.	3·49	1·22	1·08	(*a*) 0·104	65	11
5 May	3·26	1·05	0·69	(*b*) 0·050	68	34
24 May	2·50	0·94	0·59		62	37
Mean	3·08	1·07	0·79	0·029	65	26

* (*a*) is appropriate for comparisons of sowing dates. (*b*) is appropriate for comparisons of treatments and interactions.

The average effect of virus infection on the shaded plants was much less than that of shading. The yields of sugar beet sown early and late in May were reduced by 34 and 37% respectively: those of beet sown early in April were much less severely affected. The percentage of sugar in the roots was rather more severely reduced by virus infection than by shading.

Experiment II (1941). There were thirty-two plots with all combinations of the following treatments, arranged in randomized blocks of eight plots: (1) two sowing dates, 8 April and 19 May; (2) four infection dates, 25 June, 16 July, 6 Aug. and 28 Aug.; (3) four infection rates—0, 35, 70, 100%. Only the means for all rates of infection compared with no infection will be considered in this paper. The variety was Kleinwanzleben E. The plots were separated by muslin screens or cages open at the top, 6 ft. × 6 ft. × 22 in. high, so that there was very little shading of the plants. This arrangement was suggested by the behaviour of the aphides in Exp. I in failing to spread infection, except where the leaves of the plants touched one another, although they could have walked beneath the sides or over the tops of the cages. The screens greatly reduced lateral movement of the aphides from plot to plot. Only a few plants in the control plots became infected, apparently late in the season, while there was rapid spread of infection in the plots which received low infection rates, although the plants selected at random for infection were thoroughly sprayed 24 hr. after the aphides had been placed on them.

The results given in Table 2 show that virus infection caused great reduction in yield of roots and sugar, and that the reduction was greater the earlier infection took place. The average reduction for infection on 25 June was at the rate of 9·3 tons of roots, and 1·7 tons of sugar per acre. As in Exp. I the reduction of sugar yield was proportionately greater

than that of root yield because the percentage of sugar in the roots was also reduced by virus infection. This can be seen by comparing the percentage reduction figures for roots and sugar given in Tables 1 and 2.

The reduction in yield for corresponding infection and sowing dates was greater in Exp. II than in Exp. I. As cultural methods used in the two experiments were as far as possible the same, and it seems unlikely that seasonal variation in the severity of the disease could account for such a large effect, the difference is probably attributable to the effect of shading. There is other evidence that virus symptoms are less severe in low light intensities. One example has already been given in the failure of yellows symptoms to develop typically in the field in dull weather; another is that typical symptoms do not develop in the glass-houses during the winter months, though the sugar-beet plants grow moderately well.

TABLE 2. *Yield of washed roots and of sugar in tons/acre (mean of all rates of infection). Exp. II*, 1941

Date of sowing	Not infected	S.E.	Infected on 25 June	16 July	6 Aug.	28 Aug.	S.E.	Mean of all infection dates	S.E.
					Roots				
8 Apr.	15·80	0·748	6·54	9·30	12·14	14·28	0·864	10·56	0·432
19 May	13·44		4·45	5·38	10·73	12·03		8·15	
Mean	14·62	0·529	5·50	7·34	11·44	13·16	0·611	9·36	0·305
					Sugar				
8 Apr.	2·66	0·113	1·03	1·41	1·94	2·29	0·130	1·67	0·065
19 May	2·32		0·67	0·80	1·70	1·85		1·25	
Mean	2·49	0·080	0·85	1·10	1·82	2·07	0·092	1·46	0·046

Percentage reduction of yield by infection

Date of sowing	% reduction caused by infection on 25 June	16 July	6 Aug.	28 Aug.	Mean
		Roots			
8 Apr.	59	41	23	10	33
19 May	67	60	20	10	39
Mean	63	51	22	10	36
		Sugar			
8 Apr.	61	47	27	14	37
19 May	71	66	27	20	46
Mean	66	56	27	17	42

DISCUSSION

Although yellows can be found in most field crops, it is rare for more than about 1% of the plants to be affected until late in the season. Except in a few crops, therefore, present loss due to virus is probably not serious. The reasons for this are apparent from a consideration of the sources and means of dissemination of the virus in the field.

So far as is known the main source of infection is the seed crop in its second year of growth: there are probably other sources, but little is known about them and they appear to be of minor importance. All the alternative wild hosts known are annuals, and the virus is not seed transmitted. It may overwinter in mangold clamps, but these have all been used before the young root crops receive their first aphis infestation. The disease has been

observed in market-garden and allotment crops, e.g. spinach, spinach beet, seakale beet, etc., but the same holds true for these as for the mangold clamps. It is well known that seed crops may become heavily infested with *Aphis fabae*, the principal vector of yellows virus in the field, very early in the year, and that large numbers of winged migrants are produced. The seed crops often contain a high percentage of virus-infected plants, and aphides migrating from these to a root crop carry the virus with them. The type of distribution of infection, and the intensity of virus attack, seem to depend upon the interaction of three factors associated with aphis infestation: (1) the earliness of the infestation, (2) the proportion of viruliferous insects which it contains, and (3) conditions for subsequent multiplication of the insects.

(1) *Date of infestation.* With all species of aphides the date of entry into the host crops varies from year to year, and the factors which determine it are largely unknown. In the sugar-beet root crop the infestation with *A. fabae* is secondary to the production of a generation of winged migrants on some previous crop such as broad beans or sugar-beet seed crops, and thus occurs comparatively late in the growing season. This means that they can rarely produce a winged generation on the root crops at a time when very rapid and widespread increase in the numbers of infected plants could most seriously affect the yield of roots. The types of distribution of infection which are found in the root crops, the behaviour of the aphides in the two experiments described here, and the results of aphis counts in the field, all suggest that spread of virus in the root crop is by walking aphides, though the winged aphides produced at the end of the season probably introduce the virus into the steckling beds, and thus carry over infection from year to year.

(2) *Proportion of viruliferous aphides in the infestation.* Virus infection is not a necessary sequel to heavy aphis infestation, since to cause infection the insects must come from an infected source and many of the heaviest infestations probably come from broad beans, or other non-susceptible plants. On the other hand, very light aphis infestations may introduce appreciable amounts of virus if the aphides come from a nearby, or highly infective source, since each individual is capable of infecting several healthy plants, given the right conditions. The fact that aphides cannot easily be detected in the crop does not mean that suspicious looking plants are not suffering from virus infection.

Even if the aphides come from an infected source, only a few, probably, will be carrying the virus, for glasshouse tests show that comparatively few individuals in a population become viruliferous in any particular feeding experiment. How many of these individuals will still be viruliferous when they reach the root crop will depend largely upon its distance from the source of infection, since glasshouse tests also show that insects lose their infectivity more rapidly when fasting, as they would be when in flight, than when feeding. Also, the number of healthy plants which they are able to infect when they start to feed depends upon the extent to which their infectivity has been reduced by fasting. Furthermore, plants infected by insects with weak infective power produce symptoms at a slower rate than those with more efficient vectors, and so are slower in becoming centres for further spread of infection. Therefore, the farther the aphides have to fly before alighting on healthy beets, the less likely they are to cause widespread infection.

(3) *Condition for multiplication of the aphides in the crop.* Other factors influencing spread of the disease are those which regulate the aphis population in the crop. Little is known about such factors, but it suggests that different species of aphides and different crops

present different problems. For this reason we have for two years made regular counts of *A. fabae* on sugar-beet manurial trials organized by the Rothamsted Experimental Station in association with the sugar factories. These observations, so far, suggest that whether or not a crop becomes severely infested with *A. fabae* depends not so much on the number of winged migrants which originally enter it, or even on their time of arrival, as on the conditions which affect subsequent aphis multiplication. One of these is certainly the weather, but another important influence is the state of the crop.

There seems to be a prevailing opinion that aphides grow and multiply best on rapidly growing and well-nourished plants, but our experience with *A. fabae* on sugar-beet root crops is contrary to this. On the manurial experiments both agricultural salt and nitrogenous fertilizers, which increased plant growth, depressed the numbers of aphides per plot. These fertilizer effects are well established, but it is not certain whether they are large enough to be of practical importance in controlling virus disease. They are mentioned here because they suggest that good manuring helps to combat aphis attack. As there is an obligate relationship between the aphis and the virus these conditions must also affect virus incidence, but this has not yet been shown by direct virus counts, or experimentally.

From the experiments and observations discussed in this paper the conditions which encourage early and rapid spread of virus infection, and therefore greatest loss in yield, are late sowing, poor cultural conditions, and proximity of the seed crops to the root crops. Wartime conditions, involving increased seed production, shortage of labour for aphis control on the seed crops, delayed sowing, and possible shortage of fertilizers, may all tend to aggravate the situation, and convert what is at present, in most parts of the country, an endemic disease into a serious epidemic.

It is suggested that the growing of seed in the large root-growing areas should be discouraged, and that a watch should be kept for early and intense aphis infestation especially on seed crops, so that it can be brought under control by spraying or fumigation, before any serious damage is done.

Summary

The symptoms of sugar-beet yellows virus in the field vary with different meteorological and cultural conditions. Illustrations of typical symptoms are given, and some account of their appearance and distribution in the field. Infection greatly reduces the yields of roots and sugar. In experiments early infection on late sown beet caused a loss of 67 % of the root, and 71 % of the sugar yield. The loss decreased with later infection and earlier sowing date. The main source of infection and means by which the virus is carried over from year to year appears to be the sugar-beet seed crop. Proximity of the seed crop to the root crop determines the number of viruliferous migrant aphides which enter the root crop at the initial infestation. Subsequent spread of virus in the root crop is determined by the rate of reproduction and of movement of the apterous aphides. Reproduction is more rapid on late than on early sown beet, and seems to be increased by poor nutrition of the plants.

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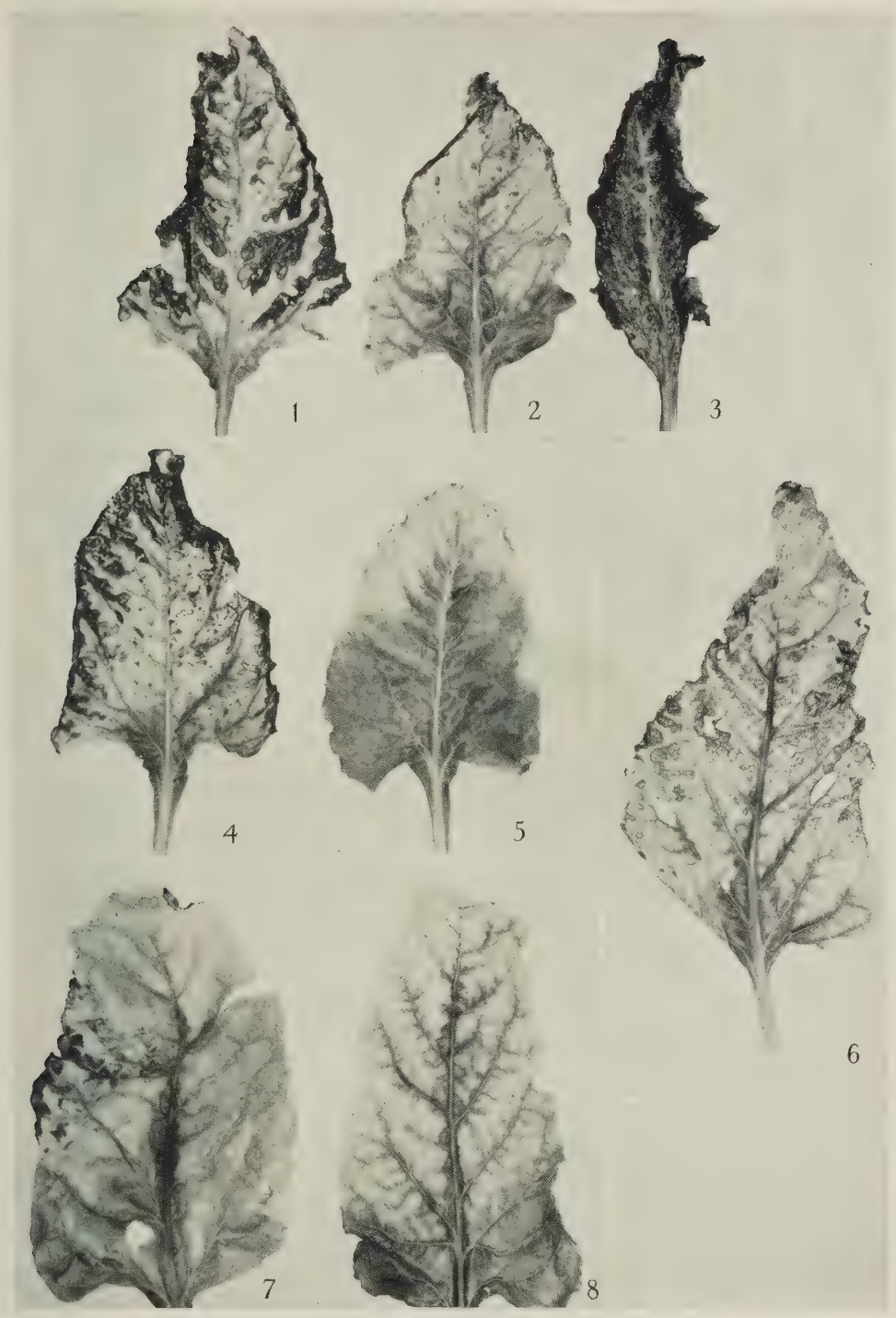

WATSON—Sugar-beet yellows virus

WATSON—Sugar-beet yellows virus

REFERENCE

PETHERBRIDGE, F. R. & STIRRUP, H. H. (1935). Pests and diseases of sugar beet. *Bull. Minist. Agric., Lond.*, **93**.

EXPLANATION OF PLATES 11 AND 12

Detached leaves from sugar-beet plants infected with virus yellows in Exp. II, 1941. All were photographed on 10 Oct., and are on the same scale: about $\times\frac{3}{7}$.

PLATE 11

Leaves from plants sown 21 May and infected on varying dates.

Fig. 1. Infected 25 June. General chlorosis except at extreme base of leaf, severe necrosis with secondary *Alternaria* infection in advanced stage.

Fig. 2. Infected 25 June. General chlorosis of most of the leaf, slight necrosis of tip and margins.

Fig. 3. Infected 25 June. Final stage of necrosis from virus and *Alternaria* infection. Most yellowed leaves degenerate into this condition if infected during a period of rapid growth. If less favourable conditions for growth of plant and virus symptoms supervene such leaves may die away and no more yellowed leaves be produced; the plant then appears to be stunted but healthy until conditions are again suitable for development of typical symptoms.

Fig. 4. Infected 16 July. Chlorosis with marginal and terminal necrosis.

Fig. 5. Infected 16 July. Leaf from plant with well-established infection but showing chlorosis only at the tip and scarcely any necrosis.

Fig. 6. Infected 16 July. General chlorosis and showing less advanced *Alternaria* infection, with small isolated clusters of fruiting hyphae instead of the usual dense blackish masses. The tapering of the tip of the leaf is a common characteristic of early virus infection.

Fig. 7. Infected 5 Aug. Chlorosis localized at the tip and on one side of the leaf, possibly the unilateral necrosis marks the site of aphis inoculation. This commonly happens in glasshouse infections.

Fig. 8. Infected 28 Aug. Chlorosis of distal and median regions of leaf. Areas surrounding mid-rib and vein remaining green. The chlorosis caused by yellows virus is typically interveinal, though severely affected leaves do not always show any green areas along the veins.

PLATE 12

Leaves from plants sown 8 Apr. and 15 May, photographed on the same scale as Pl. 11. The leaves are larger because of earlier sowing or later infection dates. (8 Apr.)

Fig. 1. Sown 8 Apr. Infected 5 Aug. Severe necrosis and tapering of the tip but little general stunting of the leaf.

Fig. 2. Sown 21 May. Infected 28 Aug.

Fig. 3. Sown 21 May. Infected during September, no stunting but terminal interveinal chlorosis.

Fig. 4. Sown 8 Apr. Infected during September. Spread of the virus is much more localized in later infections of well developed plants and often shows these rather angular patterns. Some of the chlorotic patches may mark the site of the initial infection, but others occur as scattered isolated areas, for no known reason. These isolated chlorotic lesions often start at the extreme tip of a leaf and frequently occur well away from the site of the initial inoculation, leaving the intervening leaves green and healthy looking. The green leaves and the green areas on affected leaves are generally soft and flexible to the touch, while the yellow patches are thickened and very brittle.

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A MOSAIC DISEASE OF BROCCOLI

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(With Plate 13)

A mosaic disease of broccoli has been observed in Devon and Cornwall yearly since 1936. The disease is widespread and in some seasons is very serious. As much as 75% of the plants in a field may be infected and in 1938 a field was seen so badly infected that no heads were cut from it, the crop being discarded. Broccoli is an important economic crop in this area, and as infected plants produce only a small unmarketable curd, an investigation was instituted in 1938. The spread of the disease in the field, the effect on the crop and methods of control are discussed in another paper (Caldwell & Prentice, 1942). In this paper are presented the results of experimental studies on symptomatology, host range, transmission, and the nature of the virus.

Earlier publications on the virus diseases of crucifers are largely confined to a description of symptoms, host range and methods of transmission, but more recently comprehensive studies on a number of these diseases and of the properties of the viruses responsible have been published (Hoggan & Johnson, 1935; Tompkins, 1937–9; Larson & Walker, 1939, 1941). K. M. Smith (1935), in a paper on ringspot disease of cabbage, mentioned a mosaic disease which was readily transmissible by inoculation to various crucifers but not to *Nicotiana* spp.: initial symptoms were vein-clearing followed by mottling of the lamina and some distortion. It is possible that this mosaic disease is that dealt with in this paper. Tompkins (1937) described a virus disease occurring in California, the agent of which has properties similar to those of the virus described in this paper: it is probable that it also is identical with the broccoli mosaic.

Symptoms of the disease in broccoli

In broccoli, infection, whether resulting from natural transmission in the field, or from inoculation in the glasshouse, is characterized by vein-clearing followed by vein-banding. The vein-clearing occurs from 18 to 36 days after inoculation (usually about 3 weeks) and generally is noticed at the base of the leaf so that, as usual, the symptoms appear on tissues formed after infection (cf. Caldwell, 1934). The vein-clearing may become more marked as the leaf develops, and a coarse type of vein-clearing may result in the veins becoming yellow, a condition which may persist in the oldest leaves. In the younger leaves of the plant the vein-clearing slowly passes to vein-banding, the main veins being bordered by dark green areas and the remainder of the lamina becoming chlorotic (Pl. 13, figs. 2, 3). Pronounced vein-banding is generally apparent about 6 weeks after inoculation. In some cases small, irregular, necrotic lesions subsequently develop in the chlorotic areas, appearing first as small papillae, translucent or whitish in appearance, and being more frequent on the lower surface of the leaf. Later the spots become necrotic and the colour turns to light brown (Pl. 13, fig. 6). Where vein-clearing is largely restricted to one side of the midrib and in cases where necrosis occurs, curvature of the midrib and distortion of the lamina are common.

Occasionally, a reversal of the normal vein-banding occurs, the veins being bordered by light green bands and the interveinal areas of the lamina being dark green. In other instances the combination of vein-banding of a mild type with pronounced coarse yellowing of the vein reticulum gives to the leaf a more or less uniformly mottled appearance. As happens in many virus diseases, masking of the symptoms is common. Complete masking occurs when the plants are grown at temperatures above 25° C. Partial masking of the symptoms on the later formed leaves occurs in some varieties at temperatures below 25° C.: the factors influencing this have not been fully established, but it appears to be correlated with the slow rate of growth of the experimental plants at low temperature.

The range of symptoms of infection on broccoli is wide, probably on account of the range of types found in even one horticultural variety of broccoli and also very largely to the effects of the environment. Conditions which favour steady growth also give the most marked symptoms in plants grown in the glasshouse. In the field the same variation in symptoms is found. Warm weather masks the symptoms which are intensified by a spell of cold weather. The range of symptoms is again very wide. After frost, especially spells of more than 6° F. of frost, the older leaves of infected plants fall off: this is particularly noticed in plants infected in the early autumn, the so-called primary infections (Caldwell & Prentice, 1942). Not only is the stem denuded of older leaves but the growing leaves of such a diseased plant become small and tend to bend outward with the result that the 'curd' is exposed to wet and frost and so is destroyed.

Material and methods

A stock culture was obtained in July 1939 from an infected broccoli plant (var. Sutton's Roscoff no. 2) showing vein-clearing and vein-banding, growing in the grounds of the College. Seedling plants of the same variety were inoculated with expressed sap from this plant, and serial inoculations made from them supplied the material which has been mainly used in the investigations. For purposes of comparison we have transmitted the disease by inoculation into seedlings of this variety from broccoli plants taken from several fields in Devon and Cornwall: the symptoms produced were similar to those induced by the stock culture which we had first selected as typical. In the preparation of inoculum leaves showing vein-clearing from young, recently infected plants were pounded with carborundum powder in a sterile mortar. Inoculations were made by means of a piece of cotton-wool dipped in the pulped material, additional carborundum powder being used as an abrasive. For more detailed work, e.g. resistance to ageing, temperature, etc., the pulped material was transferred to a piece of washed muslin and the juice expressed under pressure.

In experiments to determine the inactivation temperature of the virus, 0·5 ml. of the undiluted extracted juice was pipetted into thin-walled glass tubes kept at the required temperature. After being held at the desired temperature for 10 min. the tubes were rapidly cooled in tap water. For the determination of the dilution end-point, the virus extract was diluted to the appropriate concentration by means of sterile distilled water. Ageing tests were made of undiluted virus extract stored in small stoppered tubes in an incubator maintained at 22° C. In the tests on filterability the plant extract was first passed through one layer of filter paper in a Buchner funnel; a portion of the filtrate was then passed through each of a series of filter candles of progressively finer grade. The insect transmission studies were carried out in a cellophane-covered insect-proof cage in a glasshouse.

In all experiments an appropriate number of control plants were grown and none of these became diseased.

Transmission

The disease is readily transmitted to healthy broccoli seedlings by inoculation by the carborundum method and by the vector *Brevicoryne brassicae*. The first leaf symptoms appear in 18–30 days (usually in about 22 days). White fly (*Aleurodes brassicae*) is not a vector so far as our experiments go. In the spring of 1940 seed was collected from an infected

broccoli plant growing in isolation in our experimental garden. Approximately 150 seedlings were raised in the glasshouse from this seed and all were healthy at the end of 2 months. A similar trial was conducted with seed collected from an infected plant in 1941. About 500 seedlings were raised and all remained healthy after 3 months' growth. To ensure that the plants were susceptible to infection a few seedlings were inoculated with juice from infected broccoli plants. In 3 weeks two-thirds of the inoculated seedlings showed symptoms of disease. The seedlings were, therefore, not immune or specially resistant to the disease. These and other observations made over a period of years indicate that there is no seed transmission of this disease.

Varietal susceptibility

Inoculations were carried out to ascertain the susceptibility to disease of the varieties of broccoli of commercial importance in Devon and Cornwall as also of other popular varieties of broccoli and cauliflower: all of these tested were highly susceptible to infection (Table 1).

Table 1. *Varietal susceptibility*

Variety	Proportion infected	% infected
Broccoli:		
Early White	21/25	84
Knight's Protecting	18/25	72
Leamington	20/25	80
May Blossom	17/20	85
Michaelmas White	10/15	67
Satisfaction	20/25	80
Seale-Hayne: A. 1	17/25	68
B. 2	22/25	88
D.K. 7	18/25	72
D.X.S.	21/25	84
Snow's Winter White	15/25	60
Snow White	16/25	64
Sutton's Roscoff: Extra Early	19/25	76
No. 1	19/25	76
No. 2	71/85	83
No. 3	22/25	88
No. 4	21/25	84
Veitch's Self-Protecting	21/25	84
White Beauty	17/25	68
Whitsuntide	16/25	64
Cauliflower:		
All the year round	22/25	88
Majestic	17/20	85
Sutton's Superlative	24/25	96
Tozer's September Giant	24/25	96
,, October Giant	23/25	92
,, November Giant	20/25	80
Veitch's Autumn Giant	19/25	76

Host range

Under the conditions prevailing in the glasshouse the following additional species and subspecies were found to be susceptible to infection by the virus, which was recovered in each case by reinoculation to broccoli (Table 2). In each case the first symptom of infection was the same, consisting of vein-clearing sometimes followed by mild vein-banding in the case of Brussels sprouts, cabbage, and sprouting broccoli (Pl. 13, figs. 1–3). In sprouting broccoli the vein-banding was sometimes of the reversed type, i.e. with light bands along the veins and dark interveinal areas. Vein-clearing in savoys is not easy to detect with

certainty, but in doubtful cases infection was confirmed by reinoculation into broccoli. In the swede turnip and rape secondary symptoms consist of mosaic chlorosis (Pl. 13, fig. 4) with savoying of the leaves and some dwarfing of the plants. After the initial vein-clearing stage symptoms are often completely suppressed in Brussels sprouts, cabbage (all types), colewort, kale, kohlrabi, savoy and sprouting broccoli, but such 'masked' plants remained infected as was shown by reinoculation to broccoli. In general, secondary symptoms were most pronounced in plants grown at relatively low temperatures (under 17° C.), but at no time were they as conspicuous as they are in broccoli.

TABLE 2. *Host range*

Common name	Species	Proportion infected	% infected
Brussels sprouts	*B. oleracea* (L.) var. *gemmifera* DC.	15/25	60
Cabbage (Winningstadt)	,, var. *capitata* L.	6/10	60
,, (Spring)	,, var. *capitata* L.	6/10	60
,, (Red)	,, var. *capitata* L.	12/15	80
Colewort	,, var. *capitata* L.	3/8	38
Kale (Cottager's)	,, var. *acephela* DC.	13/20	65
,, (Ormskirk)	,, var. *acephela* DC.	4/10	40
Savoy	,, var. *subauda*	3/10	30
Sprouting broccoli (early purple)	,, var. *botrytis* L.	7/10	70
Kohlrabi	*B. caulo-rapa* DC.	3/20	15
Rape	*B. napus* L.	5/15	33
,,	,,	12/15	80*
Swede turnip (Monkwood)	*B. campestris* L. var. *napobrassica* DC.	10/17	60
Radish (early forcing)	*Raphanus sativus* L.	0/20	0
,, ,,	,,	6/18	33*
Charlock	*Sinapis arvensis* L.	5/15	33

* Transmission by means of *Brevicoryne brassicae*.

Natural infection of sprouting broccoli, cottager's kale, Brussels sprouts, swede turnip, spring cabbage, marrowstem kale, and common turnip was seen in the field in Devonshire, and in each case inoculation into broccoli resulted in the appearance of typical symptoms of infection by the virus of broccoli mosaic. Similar symptoms were seen in cauliflower, Brussels sprouts, swede turnip and turnip in different parts of the country, and it is believed that the virus is widespread on cultivated brassicae throughout southern Britain.

Attempts were made without success to infect other plants including common weeds of the hedgerow which might conceivably act as intermediate hosts: between ten and twenty plants were inoculated in each test (Table 3). Efforts to infect turnip by inoculation proved unsuccessful, but from our observations in the field it is clear that at least some varieties of turnip are susceptible. Infection of radishes also caused difficulty, and successful infection took place only after transmission by the vector *Brevicoryne brassicae*.

VECTORS

We have found that the virus is readily transmitted by the cabbage aphis *B. brassicae* and also by an unidentified aphis. Other species of aphis may act as vectors but under natural conditions *B. brassicae* is mainly responsible for transmission. Apterous aphides were found to be infected after feeding on an infected broccoli plant for 40 min. and such infective aphides transmitted the virus in a feeding period of 20 min. (Tables 4 and 5). Small-scale trials with alate aphides have so far given negative results.

TABLE 3. *Plants which have not proved susceptible to the virus of broccoli mosaic*

Common name	Botanical name
White Burley tobacco	*Nicotiana tabacum* L.
Turkish tobacco	*N. tabacum* L.
Night-scented tobacco	*N. affinis*
	N. glutinosa L.
	N. rustica L. var. *jamaicensis*
Tomato	*Lycopersicum esculentum*
Siberian wallflower	*Cheiranthus allionii*
Wallflower	*C. Cheiri* L.
Jack-by-the-hedge	*Sisymbrium Alliaria* (Scop.)
Sweet alyssum	*Alyssum maritimum* Lam.
	A. saxatile L. var. *compactum*
Hairy bittercress	*Cardamine hirsuta* L.
Lady's smock	*C. pratensis* L.
Shepherd's purse	*Capsella bursa-pastoris* L.
Rocket	*Hesperis* sp.
	Erysimum sp.
Candytuft	*Iberis amara* L.
Virginian stock	*Malcomia maritima* R.Br.
Ten-week stock	*Matthiola incana* R.Br. var. *annua* Voss.
Watercress	*Nasturtium officinale* R.Br.
Marsh yellow-cress	*N. palustre* DC.
	Isatis glauca
Penny-cress	*Thlaspi arvense* L.
Turnip	*Brassica rapa* L.

TABLE 4. *Transmission of the virus by the mealy cabbage aphis* (B. brassicae). *Non-infective aphides transferred to mosaic diseased broccoli for the period shown and then to healthy plants for 24 hr. (five aphides per plant)*

Time fed on diseased plants	No. of plants	No. of plants infected
0 min.	10	0
10 ,,	10	3
20 ,,	10	3
30 ,,	10	4
60 ,,	10	7
24 hr.	5	5

TABLE 5. *Infective aphides transferred to healthy broccoli for the period shown (five aphides per plant)*

Time fed on uninfected plants	No. of plants	No. of plants infected
10 min.	5	0
20 ,,	5	3
30 ,,	5	3
60 ,,	5	5
24 hr.	5	5

THE PROPERTIES OF THE VIRUS

The virus of broccoli mosaic is inactivated by heating for 10 min. at 80° C. but not at 75° C., and also after being stored at 22° C. for 8 but not for 7 days. It remains active after dilution to 1 : 2000 but not after dilution to 1 : 3000 (Table 6). Experiments on the filterability of the virus using Pasteur-Chamberland L 1 and L 3 candles were unsatisfactory, since almost complete absorption of the virus seems to take place on passage of infected juice through filter paper.

TABLE 6. *Results of experiments on temperature inactivation, ageing, and dilution. Plants used in groups of five in each experiment*

Temperature of inactivation		Resistance to ageing		Tolerance to dilution	
Temp.	Infections	Storage period	Infections	Dilution	Infections
Not heated	12/20	Zero	15/20	Undiluted	19/25
60° C. for 10 min.	10/20	1 day	6/20	1 : 10	16/25
65° C. ,,	7/20	2 days	7/20	1 : 50	7/25
70° C. ,,	7/20	3 ,,	1/20	1 : 100	4/25
75° C. ,,	2/20	4 ,,	4/20	1 : 200	4/25
80° C. ,,	0/20	5 ,,	1/20	1 : 500	2/25
		6 ,,	0/20	1 : 1000	2/25
		7 ,,	1/20	1 : 2000	2/25
		8 ,,	0/20	1 : 3000	0/25
		10 ,,	0/20	1 : 5000	0/25
		12 ,,	0/20		
		14 ,,	0/20		

OVERWINTERING OF THE VIRUS

Under commercial conditions the later varieties of broccoli are not harvested until March, April or even May, by which time the next season's seedlings are already growing. It is thus possible that infection may arise directly from the previous crop. Other farm crops, e.g. swede turnips, may also act as intermediate hosts. Frequently a month or more elapses between the end of one crop and the beginning of the next on the same farm, and in such cases it is possible that the new crop is infected from the hedgerow weeds, or, alternatively, that infective aphides live on those weeds after the removal of the old crop: we have not, however, been able to trace any common hedgerow weeds from which the virus could be recovered. *B. brassicae* is commonly found on broccoli, particularly on the plants in the seed-bed, and it is thought that this aphis acts as the vector in the field.

COMPARISON OF THE VIRUS OF BROCCOLI MOSAIC WITH THOSE OF OTHER DISEASES OF CRUCIFERS

There are few references in the literature to the occurrence in Britain of virus disease in the Cruciferae. Ainsworth (1935) reported the occurrence of the virus of cucumber mosaic in watercress. Ogilvie reported a mosaic disease of *Lunaria* which causes necrotic lesions on tobacco and which is not transmissible to broccoli. K. M. Smith (1935) reported a ringspot disease of various crucifers caused by a virus which induced mottling and necrosis in various crucifers, and necrotic lesions in tobacco. In the same paper he reported a virus causing vein-clearing and vein-banding on cauliflower which was not transmissible to tobacco. It is possible this is a reference to the broccoli mosaic virus with which this paper deals. Salaman & Wortley (1939) recorded the occurrence of potato virus '*Y*' in various Brassicae. None of these references, with the possible exception of that recorded by Smith, concerns the virus under discussion.

In the U.S.A. Tompkins and his co-workers described virus agents causing disease of cauliflower (Tompkins, 1937), Chinese cabbage (Tompkins & Thomas, 1938), turnip (Tompkins, 1938), cabbage (Tompkins *et al.* 1938), stock (Tompkins, 1939*a*), and radish (Tompkins, 1939*b*). All these, with the exception of the two viruses of stock, infected cauliflower. Larson & Walker (1939) described a mosaic disease of cabbage which was

transmissible to cauliflower on which the symptoms consist of vein-clearing and vein-banding. From New Zealand, Chamberlain (1936, 1939) described a virus similar in host range and properties to Tompkins' black king virus but considered to be a different virus. From China, Ling & Yang (1940) reported a virus disease of rape to which cauliflower was not susceptible.

From Germany, Moericke & Winter (1940) reported a virus disease of cauliflower causing vein-clearing followed by vein-banding. Of these, only Tompkins's cauliflower mosaic, Larson & Walker's cabbage mosaic and Moericke & Winter's cauliflower virosis produce vein-clearing and vein-banding on cauliflower. In addition, all but Tompkins's cauliflower mosaic differ from the broccoli mosaic under discussion in that they produced necrotic lesions on *Nicotiana tabacum*. Details of the reaction of Moericke & Winter's cauliflower virosis and of Ling & Yang's rape virus are not at present available. The symptoms of cauliflower virosis on broccoli as described by Moericke & Winter are similar to those caused by the broccoli mosaic virus under investigation. Symptoms on Brussels sprouts, cabbage and kohlrabi are described by these authors as 'severe' whereas symptoms induced by the broccoli mosaic virus are slight vein-clearing which later become masked or may develop into mild vein-banding. In either case the effects certainly cannot be described as 'severe'. Moericke & Winter's virus may prove to be allied to Larson & Walker's cabbage mosaic rather than to Tompkins's cauliflower mosaic, as is suggested by them. It would thus appear that, of the crucifer viruses described in the literature, Tompkins's cauliflower mosaic virus is the only one which does not show marked differences from the broccoli virus as regards the symptoms produced on brassicae and/or its host range.

Comparison of the properties of the broccoli mosaic virus with those of Tompkins's cauliflower mosaic virus

	Cauliflower	Broccoli
Inactivation temperature	Between 70 and 75° C.	Between 75 and 80° C.
Resistance to ageing	14 days	7 days
Dilution end-point	1 : 2000	1 : 2000

Neither virus is capable of infecting *Nicotiana* spp. The host ranges of the two viruses are almost identical and the symptoms produced on a particular host are similar. Efforts to infect shepherd's purse, ten-week stock and candytuft (which are susceptible to infection by the cauliflower mosaic virus) have been unsuccessful with the broccoli virus. This may be explained in the case of stock by the long incubation period reported by Tompkins (65–70 days); and in the case of candytuft by the low percentage of infection obtained by him (33 %).

Summary

A mosaic disease of broccoli prevalent in south-west England is described. Symptoms consist of vein-clearing followed by vein-banding and necrotic spotting. In extreme cases the plant, especially the curd, is severely dwarfed. Symptoms on other cultivated Brassicae are less severe and consist of vein-clearing which may be followed by an ill-defined vein-banding or by complete masking of symptoms. The field vector is *Brevicoryne brassicae* (mealy cabbage aphis). The virus is transmissible by juice inoculation using an abrasive such as carborundum powder. The virus resists ageing in vitro for 7 days at 22° C., and is

Fig. 1

Fig. 2

Fig. 3

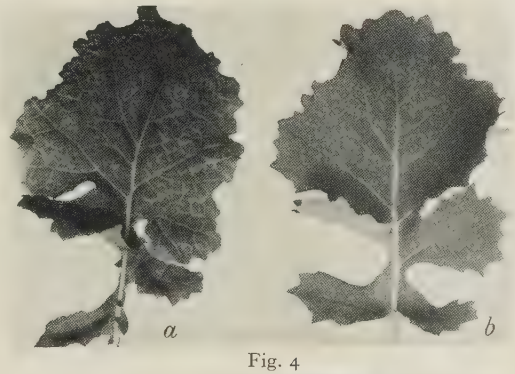

Fig. 4

Fig. 5

Fig. 6

CALDWELL AND PRENTICE—A MOSAIC DISEASE OF BROCCOLI

inactivated by heating to 80° C. for 10 min. or by dilution to a concentration of less than 1 : 2000. The similarity of host range, vector and properties indicate that the broccoli mosaic virus is identical with Tompkins's cauliflower mosaic virus. This is apparently the first crucifer virus occurring in Great Britain to be fully described.

The authors are indebted to a number of local farmers for field facilities given them in carrying out this investigation; to the Seale-Hayne Agricultural College for supplies of seed of their Roscoff Broccoli varieties; and to the Agricultural Research Council whose financial assistance made the investigation possible.

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EXPLANATION OF PLATE 13

Fig. 1. Broccoli leaves with (*a*) vein-clearing, (*b*) incipient vein-clearing (inoculated).
Fig. 2. Broccoli leaf showing vein-banding (natural infection).
Fig. 3. Sprouting broccoli showing vein-banding.
Fig. 4. Rape showing (*a*) vein-clearing and slight savoying, (*b*) healthy leaf.
Fig. 5. Cottager's kale (*a*) healthy, (*b*) vein-clearing and savoying.
Fig. 6. Broccoli showing vein-banding and necrotic spotting (inoculated).

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THE SPREAD AND EFFECT OF BROCCOLI MOSAIC IN THE FIELD

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(With Plate 14 and 1 Text-figure)

Since 1936 a mosaic disease of broccoli then first noted in this region by one of us (J. C.) has been studied. The investigation was made possible by a grant from the Agricultural Research Council, and Dr A. L. James was appointed Research Assistant. He was succeeded, on his appointment to a post in South Africa, by Mr I. W. Prentice. The present paper records the results of our investigations on the effect of the disease on the crop in the field.

Methods of investigation

Detailed studies of the distribution and spread of the disease under field conditions were made possible as the result of the kindness of a farmer in the area who put at our disposal in the first season his field of some 10 acres of broccoli. Plots were set out and each plant was marked separately on squared paper. In this way, by successive surveys, we were able to record infected plants, and to follow the spread of the disease at fairly regular intervals.

The spread of the disease in the field

In 1938 there were under investigation of Farm I, plots A, B and C, containing 400, 600 and 1000 plants respectively. They were examined first on 23–25 Sept. 1938, and showed primary and secondary infections. The former were characterized by a general mosaic marking on all the leaves of the plant, the latter showed mosaic markings only on the younger leaves. Plot A on the first survey contained 25 infected plants, primary and secondary, plot B 171 plants and plot C 196. On 23 Oct. there were twenty-three new infections in plot A, 108 in plot B, and 76 in plot C. On 13–16 Dec. there were a further 32 new infections in plot A, 74 in plot B, and 157 in plot C. The final infections in plots A, B and C were 20, 60 and 45 % respectively.

It was clear that infection had spread from diseased to healthy plants, that the spread was rapid, and that the disease had a marked effect on the quality of the curd. The infection spread from the primarily infected plants which were probably diseased at the time of planting out, i.e. were infected in the seed-bed. The seed-bed was, as is usual in Devon, put alongside a hedge at the end of the field. Since it was probable that this encouraged infection from diseased hedgerow weeds, it was suggested to the farmer that the seed-bed in the next season should be placed in the middle of a 12-acre field as far as possible from sources of infection. A second farmer was also approached with a view to extending the investigations and he kindly allowed us the use of his field for surveys. He had placed his seed-bed alongside a hedge of his field. To reduce the aphis population to a minimum the seedlings were sprayed with nicotine soap solution on 19 May and 3–6 June. Half the plants of each of six varieties at the first centre were sprayed and half the rows at the other farm. When the plants were examined on 24 June some aphides (*Brevicoryne brassicae*)

were found. The aphides actually tested were not infective when put on to healthy seedlings, though they became infective after feeding on diseased plants under laboratory conditions. Plots were laid out in such a way that the sprayed and unsprayed groups of each variety were left separate. It was intended to begin the surveys at a much earlier date than in the previous season (23 Sept.) when it was noted that some spread had already taken place in the planted-out broccoli, but the season was late and a preliminary count on 2 Aug. gave so little information that the first serious survey was made on 18 Sept. The results at the two centres are shown in Table 1.

TABLE 1. *Results of counts of diseased broccoli in season* 1939–40

Plants	Infected	New infections	
	20–23 Sept.	10 Oct.	29 Nov.
	Farm I		
Plot A: 500 sprayed	2	0	11
500 unsprayed	3	0	3
Plot B: 500 sprayed	5	2	5
500 unsprayed	5	0	5
Plot C: 500 sprayed	2	0	1
500 unsprayed	2	3	3
Plot D: 500 sprayed	28	2	15
500 unsprayed	15	7	14
	3 Aug.	18 Oct.	21 Nov.
Plot E: 1000 sprayed	9	13	11
1000 unsprayed	4	7	7
	25 Sept.	20 Oct.	29 Nov.
	Farm II		
Plot A: 700 sprayed	115	51	97
700 unsprayed	65	48	135

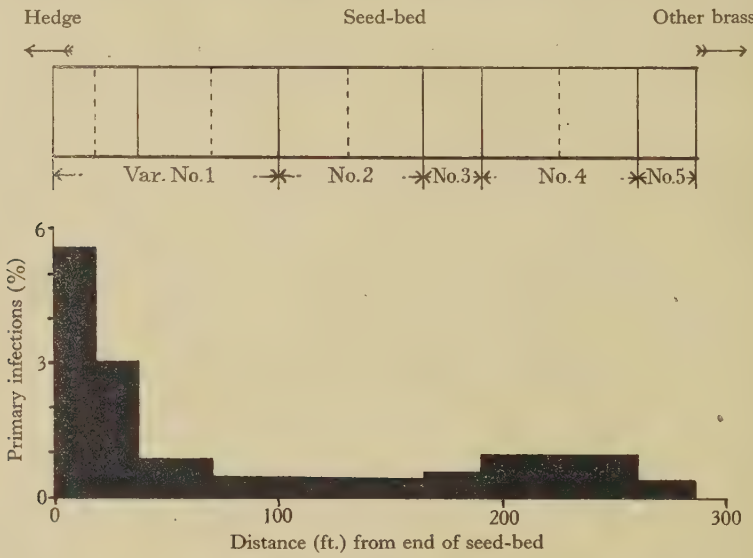
To ascertain whether any mineral deficiency might be related to the mosaic mottling, various salts were applied to different areas of the field before the seedlings were planted out. The treatments were in a randomized block, four replicates of each treatment, and four controls. Each plot was $\frac{1}{40}$ acre. The salts were magnesium sulphate applied at the rate of 30 lb./acre, zinc sulphate 20 lb., manganese sulphate 40 lb., borax 15 lb., and copper sulphate 15 lb. No effect was noted in any of the treatments. There was, further, little evidence that the nicotine soap treatment was effective in reducing the primary infection in the seed-bed. It is probable that this was due to the difficulty of getting an even layer of spray over the waxy surface of the leaves, the crowding together of the plants in the rows, and also to the extraordinarily heavy infestation of aphides characteristic of the 1939 season.

An interesting observation was that on Farm I, where the seed-bed was in the middle of the field with one end near a hedge, the largest number of primary infections was found among the plants from the rows against the hedge (see Text-fig. 1).

Frost in Jan. 1940 killed all the broccoli at both farms so that the experiments were destroyed and no evidence was obtained on the later spread or on the effect of the disease on the crop. It was, however, established that the placing of the seed-bed as far away as possible from the hedge reduced primary infections from about 30% to under 1%.

The spread of infection and the effect on the crop

In 1940 a third experimental centre was available. On Farms II and III the seed-beds were in close proximity to hedges, at Farm I the seed-bed was in the middle of the field. Spraying against aphides was not carried out at any of the centres. On Farm I there were



Text-fig. 1. Relation of amount of primary infection to position in seed-bed.

Table 2. *Numbers of infected broccoli plants at successive counts*

Plot	No. of plants	Infections 1 Oct.	New infections 6 Nov.	New infections 12 Dec.	Totals
		Farm I			
A	2800	39	127	59	225
B	1400	37	73	39	149
C	1400	24	56	30	110
D	1400	16	55	28	99
E	1400	16	26	—	—
F	1400	13	50	26	89
		30 Sept.	25 Oct.	4 Dec.	
		Farm II			
A	2800	325	851	565	1741
		Farm III			
A–E	3500	233	730	534	1497

9800 plants under observation in plots at random about the field: plots A with 2800 plants and B, C, D, E and F with 1400 each. On Farm II there was only plot A with 2800 plants, set in the middle of the field. On Farm III, which was used merely to check the amount of infection, there were 3500 plants, plots A, B, C, D and E containing 280, 320, 1080, 420 and 1400 plants respectively. The results are shown in Table 2.

Some of the earlier varieties were frosted again in this experiment in the spring of 1941. The later varieties, however, were not completely killed by frost, and much information was obtained especially at Farm II where the numbers of infected plants were for the second year very large. On Farm I the total number of infections was again very low.

A survey of Plot B on Farm I was made at the beginning of April 1941 (see Tables 3 and 4). The heads were classified as (*a*) *rotten* where the curd, and often the whole plant was in a state of decay as a result, primarily of being frosted, (*b*) *unmarketable* where the curd was damaged by frost or was very small, and (*c*) *marketable*, although all the heads might not be of first grade quality. It is clear that the disease, especially in early infections,

TABLE 3. *Percentage of broccoli plants infected at different dates (Farm I)*

New infections on	%
1 Oct. 1940	2·7
1 Nov. 1940	5·2
9 Dec. 1940	2·8
Healthy	86·7
Blank, rogues, etc.	2·6
Total	100·0

TABLE 4. *Classification of broccoli heads (Apr. 1941)*

	% of plants		
New infections on	Rotten	Unmarketable	Marketable
1 Oct. 1940	38	18	44
1 Nov. 1940	18	16	66
9 Dec. 1940	8	20	72
Healthy	1	2	97

had a marked effect on the ability of the curd to withstand frosting. This is attributed to the loss of leaves which is marked in diseased plants after frosting, and also to the tendency of the leaves of infected plants to bend backwards. Normally, the leaves of healthy plants tend to bend over and protect the 'button' or young curd. The exposure of the growing point in diseased plants to rain, snow and frost leads to its decay. The habit of healthy and diseased plants is shown in Pl. 14, fig. 3: plant (*b*) shows the effect of frost in causing premature loss of leaves and the rosette appearance of the top of primary infections. These results were confirmed on Farm II where, in plot A, the results tabulated in Tables 5 and 6 were obtained at a survey on 1 Mar. 1941.

TABLE 5. *Percentage of plants infected at different dates (Farm II)*

New infections on	%
30 Sept. 1940	11·6
25 Oct. 1940	30·4
4 Dec. 1940	20·2
Healthy	34·3
Blanks, rogues, etc.	3·5
Total	100·0

TABLE 6. *Classification of broccoli heads (Mar. 1941)*

New infections on	% of plants		
	Rotten	Unmarketable	Marketable
30 Sept. 1940	72	22	6
25 Oct. 1940	67	22	11
4 Dec. 1940	54	31	15
Healthy	31	39	30

DISCUSSION AND CONCLUSIONS

Only the experiments of the season 1940–1 could be carried to a conclusion, and even in them frost killed the earlier varieties of broccoli. We were, however, able to draw certain conclusions. Early broccoli of the Roscoff type are not suitable for growing in areas where frosts occur in the early part of the year, since each year they were killed by frost just before they were marketable. In the 1939–40 season this was hardly surprising since there was a temperature of 0° F. in the Exeter district and all the broccoli, early and late, in the area were killed, except, so far as our experience goes, in a small experimental plot on the edge of Dartmoor. Early varieties are destroyed by as little as 9° F. of frost. Since this is a not unreasonable temperature to expect in January in most areas it would appear that the early varieties should be left to Cornwall and to the very sheltered areas of south Devon.

A second conclusion is that the near proximity of hedgerow plants to the seed-bed increases the chance of infection of the seedlings: the seed-bed should be put in the middle of the field as far away as possible from the hedges. The question of primary infections is difficult. The plants infected early serve as foci of infection and also are useless from the point of view of marketing the curd. Early roguing would help in this matter and we advocate it. It would be better to leave blanks in the field if replacements cannot be made rather than have plants which produce no crop in any case and which serve as sources of infection. Roguing, however, cannot be done very early in this area, in June or July at the earliest, and the heat of these months and of August tends to prevent mosaic marking developing. Infection from these plants has often taken place before marked vein-clearing has manifested itself. Even so, roguing would help because the late-infected plants produce a higher proportion of marketable heads than do the earlier (Tables 4 and 6).

It is doubtful if spraying is of great importance: unless done very often and at considerable expense, it had little effect in checking severe infestation of aphides. Destroying the insect population of a seed-bed merely cleared the way for other aphides from neighbouring hedgerows. Where the infestation was slight the number of aphides was probably so small as to result in little difference in the crop as a whole, so far as disease was concerned.

SUMMARY

The spread and effect of mosaic disease of broccoli in the field were investigated by plotting on squared paper all the plants in large blocks. Surveys were made at regular intervals and all infected plants noted. The effect of the disease on cropping and especially the time of infection on curd production was studied in detail. Recommendations are made regarding

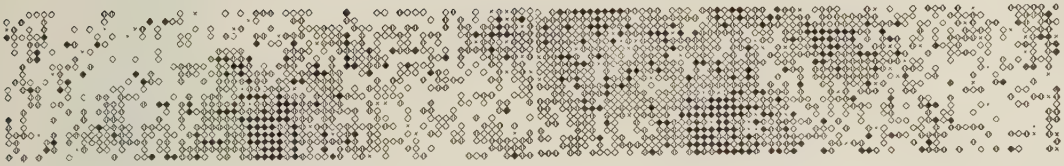


Fig. 1

Fig. 2

Fig. 3

CALDWELL AND PRENTICE—THE SPREAD AND EFFECT OF BROCCOLI MOSAIC IN THE FIELD

methods of keeping down infection, viz. (*a*) by putting the seed-bed in the middle of a large field, and (*b*) by roguing early in the season.

We acknowledge with gratitude a grant from the Agricultural Research Council without which the investigation would not have been possible. Sincere thanks are due to the farmers who so kindly put their fields at our disposal and allowed us to set out plots on their farms, often at some inconvenience to themselves.

EXPLANATION OF PLATE 14

Fig. 1. Plot A, Farm II.

Fig. 2. Plot A, Farm I. ◆ = 1st infection; ◈ = 2nd infection; ◇ = 3rd infection; × = blanks; spaces = healthy plants. Each plot = 20 × 140 plants.

Fig. 3. Broccoli mosaic: (*a*) healthy plant, (*b*) primary infection.

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INVESTIGATIONS ON THE BIOLOGY AND CONTROL OF THE CARROT FLY (*PSILA ROSAE* F.)

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(With 3 Text-figures)

A detailed investigation of the biology and of measures for the control of the carrot fly was commenced in April 1941. Special consideration was given to large-scale measures of control. These were aimed at the destruction of the adults, as previous experiments on the prevention of egg laying by deterrents (e.g. naphthalene) and on killing the eggs in the soil (by 4% calomel dust) had given disappointing results. To assist correct timing of the treatments detailed observations were made on the times of emergence and abundance of the flies in the field.

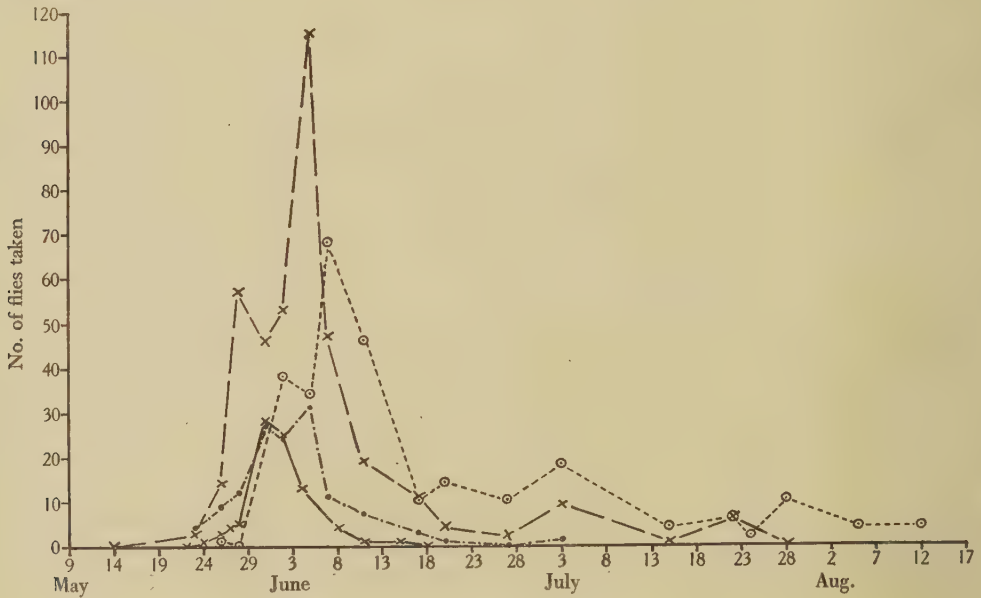

Fig. 1. Carrot fly: 1st generation. Emergence and abundance of adults in Cambridgeshire.

× — — — × No. of flies emerging in 9 cages (Hall Farm, Mepal, Chatteris).
⊙ - - - - - ⊙ No. of flies taken from dykeside in 100 sweeps (Hall Farm, Mepal, Chatteris).
× ——— × No. of flies emerging in cage over clamped carrots (Cambridge garden).
• -·-·-·-· • No. of flies emerging in 9 cages (B. Hadder, Mepal, Chatteris).

Emergence and abundance of carrot fly, 1941

Emergence of the first generation

Two sets of nine muslin cages were erected on fields at Chatteris (Isle of Ely) in early May. The carrots in both fields in 1940 were fairly heavily attacked by carrot fly. Another cage was erected on the site of a clamp in a Cambridge garden. The cages were examined at frequent intervals and the numbers of flies are recorded graphically (Fig. 1). Flies were

also obtained by sweeping the vegetation with nets. The greatest numbers were taken on fields which had grown carrots in 1940. In the Chatteris district, sweeping and observation showed that the vast majority of the flies were in the vegetation of the dike-sides, probably because crops at this time were small and afforded little protection. By periodically carrying out a given number of sweeps on the same vegetation an estimate of the abundance of the flies was obtained. The results for a dike-side adjoining land cropped with carrots in 1940 are shown in Fig. 1. A sharp decrease in the number of flies taken occurred in early June, probably directly correlated with a decline in the rate of emergence which preceded it. This decline coincided with a change of weather, viz. a considerable fall in temperature and much rain.

Emergence of the second generation

Nine muslin cages were erected over carrots on the headland of Cage Field, Hall Farm, Mepal, on 22 July. The crop was sown in mid-May and incurred some damage by the latter part of the first generation. Emergence commenced on 12 Aug. and continued until 16 Oct. Major peaks in the rate of emergence occurred in the last week of August and at the beginning of the second week of September, while a minor peak occurred in the first week of October. Between the emergence of the first and second generation small numbers of carrot flies could be swept from carrot fields. In late July two cages together covering 1 sq. yd. were erected over hemlock (*Conium maculatum*) plants in a rickyard at Hall Farm. During the period 22 Aug.–16 Sept., forty-eight *Psila rosae* were obtained from these.

Experiments on the control of carrot fly, 1941

Control of the first generation

Experiments carried out in the U.S.A. (Whitcomb, 1929, 1938) showed that carrot-fly adults are killed by derris powder, and indicated that a good control of the first generation could be obtained with two applications of derris powder of 0·6–1·0% rotenone content. Two fields of early carrots in the Chatteris district were each given two applications of derris powder of 1·0% rotenone content, applied with a power duster travelling at about 1 mile/hr. and trailing a 100 ft. long drag sheet. Treatment was carried out in early morning or late evening during cool and still air conditions. Some delay in carrying out the dustings resulted from unsuitable weather conditions. The whole of each field and the adjoining dike-sides were treated, approximately 60 lb. of dust/acre being used at each application. The smaller field (5 acres) was treated on 3 June and 16 June, and the larger field (11 acres) on 5 and 6 June, and again on 13 and 14 June. Flies were common on both fields before the first treatment, especially in the dike-sides. The day following this treatment a few dead flies were found on the field and many on the headlands under the overhanging vegetation. Of the flies swept from the treated vegetation at this time 50% were dead the next day. Sweeping about 1 week later, however, revealed that flies were again as numerous on the treated fields as in the neighbouring untreated ones. Counts of eggs made at this time showed there was little difference in the numbers present in both sets of fields. Later sweeping and observation suggested that re-infestation from neighbouring fields was occurring shortly after each treatment. During the emergence period of the first generation, dike-sides and roadsides in the Chatteris area were swept and carrot flies were found to be widespread and occasionally the commonest insect taken. This suggests that a local artificial

decrease in the numbers of carrot fly is liable to be swamped rapidly by the influx of flies from the large moving population in the neighbourhood. This might be offset by frequent applications if the cost of each is low. The derris treatment is too expensive for this purpose.

Many eggs were laid by the first generation, and it was estimated that approximately 50% hatched. Samples of carrots were taken in mid-July from the treated fields and from neighbouring untreated ones. Very slight damage from carrot fly larvae was found, the attack on early carrots in this district being negligible. This failure of a promised attack to materialize was probably due to the very dry soil conditions during the last fortnight of June and the first fortnight of July.

Control of the second generation by a poison bait

Preliminary experiments showed that flies of the first generation could readily be killed by spraying the dike-side vegetation with a poison bait mixture of 0·8% sodium fluoride and 2·5% cane molasses in water. This method of control was tested on a field scale against the second generation. Sweeping again showed that, as with the first generation, the vast majority of flies were to be found on the headlands and dike-sides of the carrot fields. Six fields of main crop carrots, in districts known to be infested, were chosen for treatment. On the five smaller fields (4, 4, 8, 11 and 12 acres respectively) the headlands and dike-sides only were treated. The remaining field of 19 acres was similarly treated, but in addition strips about 3 yd. wide were treated across the field at 15 yd. intervals. Ten applications were made from 19 Aug. to 29 Sept.; the interval between successive treatments varied from 3 to 7 days, being shortest during the peak emergence period. The bait solution was applied as a coarse spray by a tractor-drawn pressure sprayer. A strip approximately 3 yd. wide was treated behind the machine, and after the first application an adjustable spray lance was fitted and directed into the dike-side and neighbouring vegetation. The two small treated carrot fields at Hall Farm, Mepal, grew carrots in 1940. These were badly attacked by carrot maggot. There were no other carrots in the vicinity with which the present crops could be compared. The remaining fields, situated in pairs in two other localities, bore carrot crops occurring in a normal rotation. Both adjacent to and in the neighbourhood of these were similar crops which were used for comparison.

An estimate of the number of flies present in one treated field (Cage Field) was obtained by carrying out a definite number of sweeps in different parts of the field and in the adjoining dike-sides and potatoes. The sweeping was carried out in suitable weather conditions at intervals during late summer, autumn and early winter. The numbers of flies so obtained and dates on which spraying was carried out are shown graphically in Fig. 2. A summary of these data, together with temperature and weather records, are given in Table 1.

In five cases out of seven covering the peak emergence period, treatment appears to have brought about a sharp decrease in the abundance of the flies. For example, sweeping carried out under similar weather conditions at 10.30 a.m. on both 26 and 27 Aug. yielded thirty-six and ten flies respectively. Spraying at 11 a.m. on 26 Aug. probably brought about this decrease. Similar decreases in abundance occurred after spraying on 30 Aug., 2, 9 and 13 Sept. No observed decrease in flies resulted from sprays applied on 19 Aug. and 6 Sept. That of 19 Aug., the first of the series, was experimental in nature and was followed by rain the same evening, about 3 hr. after treatment. On 6 Sept. treatment was

carried out at approximately 10.30 a.m. in cool misty conditions. It is probable, therefore, that the first failure resulted from washing off, but no satisfactory explanation is offered concerning the second.

On other treated fields it was not possible to follow by sweeping the variation in numbers of flies on account of the small numbers taken. Sweeping showed, however, that in these fields they were considerably fewer than in untreated carrot fields in the vicinity. A small number of dead flies was found in the treated fields but not in the untreated.

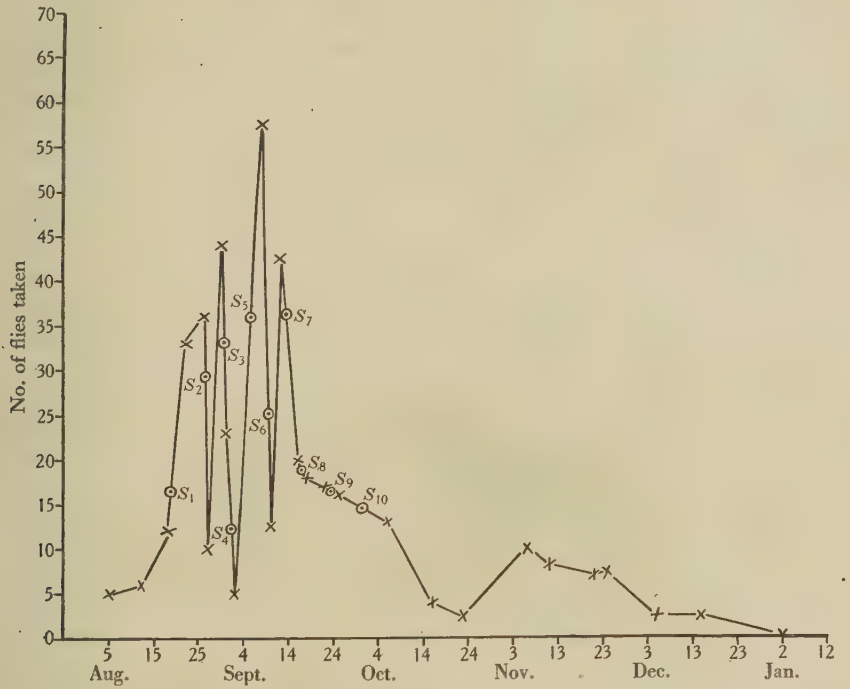

Fig. 2. Carrot fly: 2nd generation. Abundance of adults on a treated field. (Hall Farm, Mepal, 1941.)

×———× No. of flies taken in 25 sweeps. ⊙S Application of poison bait.

Observation in the laboratory and in the field showed that the flies take the fluoride-molasses solution readily and are rapidly killed by it. In 'Cage' field, which was part carrots and part potatoes, flies of the second generation were found to be most abundant on the rows of carrots next the potatoes, and in the first three rows of potatoes adjoining. Counts were made in this region of the number of flies killed by various spray applications. Transverse sections, 1 yd. wide, were taken across the edge of the carrots and into the potatoes and the number of dead flies in the inter-row spaces were counted (Table 2).

Sections along this strip showed that the distribution of dead flies was fairly uniform along its whole length (425 yd.). With the carrot and potato rows spaced at 14 and 24 in. respectively, the width covered by the sections was approximately 16 ft. Using these dimensions it was calculated that the number of flies killed on this strip by sprays applied

TABLE 1. *Summary of treatment and sweeping data with temperature and weather records (Cage Field, Hall Farm, Mepal, 1941)*

Application of poison bait	Sweeping	Av. no. of flies taken in 250 sweeps	Max. day shade temp. ° F.	Weather conditions at time of sweeping
	12 Aug. a.m.	6	65·3	Cool, dull
	18 ,, a.m.	12	68·2	Sunny periods, cool
19 Aug. p.m.			67·2	
	22 ,, p.m.	33	67·5	Sunny, warm
	26 ,, 10.30 a.m.	36	66·6	Dull, cool
26 ,, 11 a.m.			66·6	
	27 ,, 10.30 a.m.	10	65·8	,,
	30 ,, 10.0 a.m.	44	67·9	,,
30 ,, 10.30 a.m.			67·9	
	31 ,, 3 p.m.	23	71·5	Sunny, warm
2 Sept. 9.30 a.m.			77·6	
	2 Sept. 3 p.m.	5	77·6	Very sunny, warm
6 ,, a.m.			71·0	
	8 ,, p.m.	57·5	64·2	Dull, warm
9 ,, a.m.			72·0	
	10 ,, p.m.	12·5	66·2	Sunny periods, warm
	12 ,, p.m.	42·5	61·4	Dull, warm
13 ,, a.m.			63·5	
	16 ,, p.m.	20	60·4	Sunny, warm
17 ,, a.m.			65·8	
	18 ,, a.m.	18	66·1	,,
	22 ,, a.m.	17	65·8	,,
22 ,, p.m.			65·8	
	25 ,, p.m.	16	75·2	Dull, warm
29 ,, p.m.			64·2	
	6 Oct. p.m.	13	72·7	Sunny, warm

TABLE 2. *Distribution of dead carrot flies (killed by poison bait) in carrots and potatoes (Cage Field, Hall Farm, Mepal, 1941)*

		No. of dead flies collected in inter-row spaces 1 yd. long			
		Max.	Min.	Mean	No. of samples
Potato rows:	4	0	0	0	1
	3	7	5	5·7	4
	2	26	2	12·7	6
	1				
Carrot rows:	1	27	2	15·5	10
	2	21	1	7·7	11
	3	4	0	2·3	3
	4	8	2	4·6	5
	5	6	4	5·0	2
	6	9	5	7·0	2
	7	3	2	2·7	3
	8	3	3	3·0	1
	9	—	—	—	0
	10	1	1	1·0	2
	11	—	—	—	0
	12	0	0	0	1
Total		115	27	67·2	51

on 26, 30 Aug., 2 and 9 Sept. was approximately 8300, 33,300, 27,600 and 28,200 respectively. Of these, some 64% occurred in the following three inter-rows, between potato rows 1 and 2, carrot rows 1 and 2, and in a strip 18 in. wide between the two crops. A large number of flies were also killed on this strip by sprays applied on 13 and 17 Sept. No counts of dead flies were made on the other edges of this field but on several occasions after spraying they were noted to be common there. Dissection of the dead flies showed that a high percentage were immature and mature females.

Estimates of the degree of attack in the treated and neighbouring untreated fields were made in late October and early November. Samples of carrots were taken both from the headlands and from the remainder of the field and the results shown in Table 3 were obtained. In all the treated fields the attack was lower than in the untreated fields. Taking the number of mines for 100 carrots as a measure of the attack, the average infestation on the untreated fields was from 133 to 217% higher than on the treated fields.

TABLE 3. *Control of carrot fly. Second generation* 1941

Treatment.—Ten applications sodium fluoride and molasses. Samples taken 16, 20, 27 Oct. and 7 Nov.

Sample	Average % Clean	Slight	Moderate	Unsaleable	Av. mines per 100 carrots
1. District 1. Chatteris-Mepal Road					
(*a*) Two treated fields (total 20 acres)					
Headland	81	15	3	1	24·0
Mid-field	91	8	0·5	0·5	11·0
(*b*) Four untreated fields (total 21 acres)					
Headland	63·5	23	7·2	6·2	54·4
Mid-field	79·5	15	2·8	2·8	27·0
2. District 2. Horseway, Chatteris					
(*a*) Two treated fields (total 31 acres)					
Headland	86·5	9·5	4	0	18
Mid-field	90·5	7·5	2	0	11
(*b*) Two untreated fields (total 33 acres)					
Headland	66	18·5	10	5·5	63·0
Mid-field	85·5	8	4·5	2	29·0

Control by grass cuttings and 4% calomel dust* 1941

A small-scale experiment on the control of the second generation of carrot fly was carried out at the Entomological Field Station, Cambridge. A stump-rooted type of carrot was sown on 28 Apr., but owing to dry soil conditions did not germinate until a month later. Six treatments were included with six replicate plots of each; these were randomized. The following treatments were carried out:

Treatment 1. Four dressings of grass cuttings 16 June, 6 Aug., 21 Aug. and 11 Sept.

" 2. Three dressings of grass cuttings 6 Aug., 21 Aug. and 11 Sept.

" 3. Two dressings of grass cuttings 6 Aug. and 21 Aug.

" 4. One application of 4% calomel dust at 1 lb./40 row-yd. 6 Aug., followed immediately by grass cuttings on top of this. Grass cuttings alone 21 Aug. and 11 Sept.

" 5. One application of 4% calomel dust at 1 lb./40 row-yd. applied on top of grass cuttings, 6 Aug. Grass cuttings alone 21 Aug. and 11 Sept.

" 6. Control (untreated).

* See Davies, W. M. (1931).

The grass cuttings were put along the rows close to the carrots at about the same rate on each plot at each application. In late summer the foliage on the plots receiving grass cuttings was more vigorous and much darker green than on those plots receiving no cuttings (control). At lifting, however, no difference in the size of the carrots on the different plots was noted.

All plots were sampled on 30 Oct., twenty carrots being taken at random from each plot. These were washed and graded and the number of mines per carrot counted. The results were as follow:

Treatment no.	% unattacked	% heavily attacked (unsaleable)	Total no. of mines in 100 carrots
1	52·5	12·5	75·8
2	53·5	11·8	103·3
3	52·5	17·5	90·8
4	58·5	14·2	67·5
5	45·0	15·8	95·8
6	34·2	34·2	176·6

Statistical analysis of the percentage of unattacked carrots showed that treatments 1, 2, 3 and 4 were significantly better than the control (6) with treatment 4 standing out as the best. There was no significant difference between treatments 5 and 6. When the number of mines was considered treatments 1, 2, 3, 4 and 5 were all better than the control, with 4 again the best.

Observations on the biology and distribution of the carrot fly

Distribution of flies in carrot fields

By sweeping and observation it was found that carrot flies of both the first and second generations were far more abundant on the edges of carrot fields and in the adjoining dike-sides or hedges than the remainder of the carrot field. It seems that the flies choose these positions on account of the shelter obtained there. During high wind or rain, few flies were taken by sweeping but they were then found sheltering in the vegetation. For this purpose, stinging-nettle, dead-nettle and potato foliage were especially favoured. Flies were also taken from other broad-leaved plants and from low-growing bushes of elder and elm. Few were found on grasses. The distribution of flies across a strip of carrots into the adjoining potatoes and dike is shown in Fig. 3. The data were obtained in late August and September by sweeping.

Distribution, position and numbers of carrot fly eggs

Observations and counts made on carrot fields in June showed that eggs were most common around the edges and fewest in mid-field. On one field the average number per foot of row on the outside rows and 13, 15 and 100 yd. from the edge was 20, 16, 11 and 0 respectively. The uneven distribution of both flies and eggs over carrot fields furnishes an explanation for the heavier maggot damage found on the edges as compared with the remainder of the field (see Table 3).

The eggs are usually laid in cracks or under small lumps of soil, about $\frac{1}{8}$–$\frac{1}{4}$ in. below the surface. Occasionally they are found on the surface or stuck to the leaf bases or to the crown of the carrot. Most eggs occurred within $\frac{1}{4}$ in. from the plant, but they were also

found up to $1\frac{1}{2}$ in. away. They were usually found singly or in groups of two or three but clusters containing as many as seven occurred.

The number of eggs laid on young carrots appears to increase with the size of the tops or with some other factor which increases with age. On carrots sown at different dates on adjoining plots the average number of eggs per foot of row in mid-June was as follows:

Date of sowing	No. of rough leaves	Av. no. of eggs/ft. of row
20 Mar.	6	8·5
8 Apr.	5	6
15 ,,	4	2·5
22 ,,	3	1·5
29 ,,	2	0
6 May	1	0
27 ,,	Cotyledons only	0

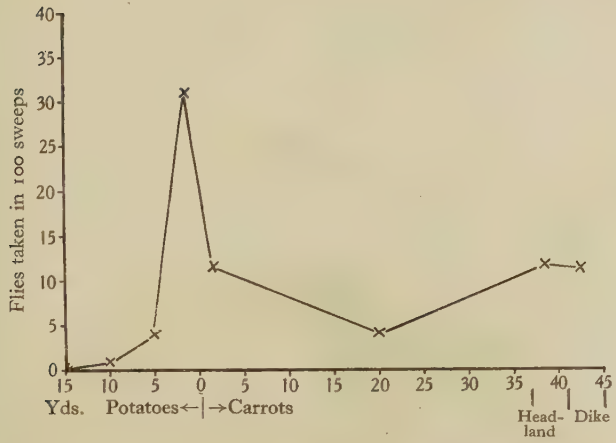

Fig. 3. Distribution of carrot fly: 2nd generation (Hall Farm, Mepal, 1941). Relative abundance in carrots and adjoining dike-side and potatoes. Total number of flies in 100 sweeps equals average of five records.

In the field eggs were found once near thickly sown carrots with two rough leaves. Carrot flies were abundant on this occasion and ninety-two eggs were found around a one-year-old carrot on the same field. Eggs were not found around carrots with one rough leaf or with cotyledons only.

Egg laying by carrot flies emerging in autumn and early winter, 1941

On 6 and 11 Nov., twenty-two flies were taken by sweeping in a carrot field and from these eighty-one eggs were obtained in captivity. These were kept at room temperature and 37% hatched. Flies from the same source, taken in the latter half of November also laid many eggs but these failed to hatch. In this case both flies and eggs were kept in an unheated insectary. Flies taken in December did not lay.

Evidence suggesting that egg laying and hatching occurred also in the field in autumn and possibly early winter was obtained from the examination of carrots at this time. Very small larvae, considerably less than half the full size, were then obtained. These comprised 8·8% of the total larvae present on 27 Oct. 1941, 0·9% on 11 Nov. 1941, and were last found on 15 Dec. 1941.

Duration of the larval stages in autumn and early winter

According to Smith (1922) all the maggots of the second generation pupate about the end of September. On one occasion some were found feeding at the end of October. In the eastern counties observations and sampling show that the vast majority of the larvae do not leave the carrots to pupate until late winter and early spring.

The number and size of the larvae taken from a large number of carrot samples in the autumn and early winter 1941 progressively increased until December. In January and February their numbers decreased and most were then fully grown. This trend in numbers and in size was also found in the larvae taken from clamped carrots. Two sets of the data obtained are given in Table 4.

TABLE 4. *Numbers and size of larvae taken from unlifted and clamped carrots (Cambridge 1941–2)*

	Unlifted carrots		Clamped carrots	
Date of sampling	(*a*) Total larvae in 100 carrots	(*b*) No. of larvae more than half-grown %	(*a*) Total larvae in 100 carrots	(*b*) No. of larvae more than half-grown %
4 Nov. 41	120	60·8	76	60·5
17 „ 41	101	63·5	94	58·5
1 Dec. 41	231	80·2	78	66·7
16 „ 41	293	92·1	60	90·0
1 Jan. 42	175	88·0	63	87·3
26 „ 42	163	95·1	57	96·5
2 Mar. 42	79	96·0	30	100·0

In March 1941 soil was taken from beneath a clamp and from the carrots in it. Each sample weighed about 8 lb. Larvae and pupae were removed from these by sieving. On the same date 50 carrots were taken at random from the clamp and the number of larvae in these ascertained. This sampling was repeated again in April. The following data were obtained:

	Soil from under the clamp		Soil from the carrots		
Date of sampling	(*a*) larvae	(*b*) pupae	(*a*) larvae	(*b*) pupae	No. of larvae in 50 carrots
18 Mar. 41	59	38	82	35	7
5 Apr. 41	3	36	1	49	2

All the larvae taken on both occasions appeared to be fully grown. In unlifted carrots in the Chatteris area on 20 Mar. 1941, larvae were present in fair numbers.

Intensity of carrot-fly attack in relation to date of sowing

Carrots were sown at weekly intervals on adjacent plots from 8 Apr. to 5 Aug. inclusive. At each sowing three types of carrot were included, a stump-rooted type (Clucas's 'Early Market'), a James's Intermediate type (Clucas's 'New Model') and a long parsnip-rooted type (St Valery). The carrots were lifted and graded in December. On sowings up to 24 June inclusive the attack was heavy and of about the same intensity on all sowings. Carrots sown after this date were progressively less damaged. The last two sowings, however (29 July and 5 Aug.), although only slightly attacked, were too small to be valuable com-

mercially. On all sowings there was little difference between the degree of attack on the three types of carrot.

Effect of carrot-fly attack of sowing onions with carrots

The possibility that carrots grown in close proximity to onions obtain some protection from carrot fly was investigated at Cambridge. On one plot alternate rows of onions and carrots, 1 ft. apart, were sown in March. On another, approximately 20 yd. away, carrots only were sown. The variety, date of sowing, and seed rate of the carrots were the same on both plots. Subsequent cultural treatment was also similar. In June, carrot-fly eggs were found to be about equally common on both plots. In October, the carrots on both were examined and the following attack was recorded:

	% clean	% slight	% moderate	% heavy (unsaleable)	Total no. of mines per 100 carrots	No. of roots examined
Carrots with onions	90·0	6·5	2·6	0·9	14·7	339
Carrots alone	87·5	9·9	1·8	0·8	15·0	272

As the damage on both plots was very similar, it appears very doubtful if onions exercise any deterrent action upon carrot fly.

TABLE 5. *Deterioration of unlifted early main-crop carrots (Chatteris, 1941)*

Date of sampling	Clean %	Slightly attacked %	Moderately attacked %	Heavily attacked (unsaleable) %
12 Aug. 41	91	7	2	0
27 ,, 41	86	8	6	0
10 Sept. 41	84	10	6	0
25 ,, 41	72	20	8	0
27 Oct. 41	40	28	20	12
11 Nov. 41	30	26	20	24
15 Dec. 41	28	24	23	25

Rate of deterioration of carrots

As the carrot-fly larvae increase in size so the area of the root mined and the depth to which the larvae penetrate increase. The maximum damage to the host crop will not therefore be attained until all the larvae have left it. To follow this process of crop deterioration a number of carrot fields and plots were sampled at intervals throughout autumn and early winter. The samples were washed and graded according to severity of attack into clean (unattacked), slightly, moderately and heavily attacked (unsaleable). The results obtained for a 4-acre field in the Chatteris area are given in Table 5. The samples were taken from the general field (excluding the headlands).

Deterioration was most rapid in October and early November. The samples taken in November give a very different picture of the condition of the crop from that obtained in September.

In another field in the same locality, the deterioration over the same period was very much less. In September, 94% of the carrots were clean, with the remainder slightly attacked, whilst in mid-December 77% were clean and 2% only unsaleable. The probable explanation of these differences lies in the fact that the first mentioned field was sown in May and suffered some first generation attack, whilst the second field was sown in June and

escaped this attack. Following this, second generation adults were very abundant on the first field and comparatively scarce on the second.

The deterioration of unlifted main-crop carrots and of the late (July) sown crop has been followed also at Cambridge. In these, deterioration was most rapid during the latter half of October and throughout November.

A comparison of rate of deterioration in clamped and unlifted carrots was also made. The results shown in Table 6 were obtained at Cambridge; similar data were obtained at Chatteris. The carrots clamped were of the same sowing and variety as those unlifted and taken from land adjoining these.

TABLE 6. *Rate of deterioration of clamped and unclamped carrots (Cambridge)*

	Unlifted carrots			Clamped carrots		
Date of sampling	(*a*) Clean %	(*b*) Unsaleable %	(*c*) No. of mines per 100 carrots %	(*a*) Clean %	(*b*) Unsaleable %	(*c*) No. of mines per 100 carrots %
4 Nov. 41	42	28	138	45	18	113
17 „ 41	33	29	171	40	21	144
1 Dec. 41	16	39	264	35	23	153
16 „ 41	15	41	279	36	19	137
1 Jan. 42	16	42	255	37	18	136
26 „ 42	18	53	303	39	24	130
2 Mar. 42	15	56	291	40	19	125

Table 6 shows that, over a period of about 4 months the number of unsaleable carrots in the unlifted crop increased by 100% as compared with little or no increase in those clamped. In the unlifted carrots extensive deterioration is seen to be accompanied by a considerable increase in the number of mines. This appears to be a result of migration of the larvae from carrot to carrot. On many occasions a large larva was found with the hind end protruding from a mine which was shorter than the larva itself. Such mines, with little doubt, represented the beginning of a new region of attack by a migrant larva. In the clamps little migration appeared to occur as the number of mines did not markedly increase during storage.

The clamping of carrots in early winter is recommended not only as a precaution against destruction by severe frosts but also to reduce the rate and extent of deterioration of crops damaged by carrot fly. Furthermore, during the process of lifting, topping and clamping a considerable number of larvae are lost from the carrots. Evidence was also obtained that attacked carrots are more susceptible to frost damage than those not attacked.

Host plants

The adults of the first generation were seen feeding on the flowers of wild chervil (*Anthriscus sylvestris*) and on those of hemlock (*Conium maculatum*). Such feeding does not appear to be an essential preliminary to egg laying as small numbers of fertile eggs were obtained, both in the laboratory and in the field, from flies which had been enclosed with moist soil and seedling carrots from the time of emergence. The length of life and number of eggs laid, however, are probably both greatly increased by feeding. The commonest larval host is the cultivated carrot. Where early and main crop carrots are grown in the same locality both generations of the fly find admirable conditions for propagation. Both

generations also attack the roots of hemlock (*Conium maculatum*) which is very common on dike-sides, in rickyards and on other uncultivated land in fen districts. It behaves as a biennial and the larvae which mine in the cortex of the thick tap root appear to be identical with those taken from carrots. In two cages, together covering approximately 9 sq. ft., placed over hemlock plants in a rickyard, 48 adults emerged from 11 Aug. to 16 Sept. inclusive. These were identified by J. E. Collin as the carrot fly *Psila rosae*. This is a new host record. Celery is also attacked. In June, numbers of eggs were found around plants in the Chatteris area. The larvae mine in the stem and leaf stalks and may cause considerable damage. As many as eight larvae were taken from one plant in November. No signs of attack were found on the wild carrot or other wild umbelliferous plants.

Survey of carrot fly infestation in Norfolk and west Suffolk

In the last week of August and the first week of September, during the peak emergence period of the second generation, a survey was made to estimate the abundance of flies in the carrot fields in Norfolk and west Suffolk. As few flies were found, sweeping was confined to the headlands. In each field 100 sweeps with a net were made. In the Swaffham, Fakenham and Heacham districts of Norfolk, twelve flies were taken from twenty-four fields of a total acreage of approximately 1050. One fly was taken from land newly broken from breck and not known to have been cropped with carrots before. In the Norwich and Thetford districts four flies were taken on eleven fields of total acreage 116. At Risby, West Suffolk, nine flies were taken in two fields totalling 60 acres, but here conditions for sweeping were poor.

In late September and early October random samples of carrots were taken from seventeen fields in Norfolk. From fields of 10 acres or less fifty carrots were taken, and from larger fields 100 carrots. The attack was very slight, 0·53% of the roots being only slightly damaged, with an average of 0·35 maggot mines per fifty carrots. In samples taken from nine fields (total 76 acres) in the Chatteris area at about the same time the attack was as follows:

	Percentage damaged			
Undamaged	Slight	Moderate	Unsaleable	Av. no. of mines per 100 carrots
84	11·2	3·1	1·7	22·6

On 9 Dec. samples were taken from four fields at Risby (totalling approximately 114 acres) and the attack was as follows:

	Percentage damaged			
Undamaged	Slight	Moderate	Unsaleable	Av. no. of mines per 100 carrots
84·6	9·7	4·0	1·7	26

In the Chatteris area carrots have been widely grown for many decades; at Risby, however, they are a comparatively new crop, only having been grown extensively for about the last 10 years. During this time very close carrot cropping has been practised; on one field carrots have been taken 3 years in succession. This system appears rapidly to be building up a damaging carrot fly population.

Summary

Observations were made on the emergence and abundance in the field of adults of the first and second generations of the carrot fly. Experiments to control the first generation on early carrots by derris dust were not successful. Large numbers of second generation flies were killed by spraying the dike-sides and headlands with a poison bait: this reduced the crop damage in the treated fields. Some control in small scale experiments was obtained by placing grass cuttings with and without 4% calomel dust along the rows. The adults of both generations were found in greatest numbers on the headlands and dike-sides of carrot fields. Eggs were most common on the headlands of carrot fields. Observations were also made on the position and numbers of eggs in the soil and their occurrence in late autumn. Few larvae appear to leave the carrots to pupate until late winter and early spring. Carrots sown at weekly intervals from April until late June were attacked to a similar extent: on carrots sown after this date the attack progressively decreased. No decrease in infestation resulted from sowing onions with carrots. Deterioration through maggot attack in unlifted carrots was most rapid in October and November. In clamps, deterioration was less rapid and extensive than in unlifted carrots. The flies feed on the flowers of wild chervil and hemlock: hemlock is also a host for the larvae of both generations. A survey of carrot fields in Norfolk in 1941 showed that flies of the first generation were scarce. The attack which followed was very slight. In west Suffolk the number of flies present and the damage recorded later were much greater.

The writers are indebted to the Agricultural Research Council for a grant which has financed this work and to Mr A. S. Rickwood for providing facilities for experiment and observation. Thanks are also due to Messrs Pest Control, Ltd., for carrying out the experimental treatments.

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Added to proof, 19 *Oct.* 1942. In 1942 observations on the biology and distribution of the adults confirmed those of the previous year. In field scale trials in two localities the molasses and sodium fluoride bait again resulted in high mortality of flies and also in a marked reduction of the maggot damage to the crop.

CRAMBUS HORTUELLUS Hb. AS A GRASSLAND PEST

By H. W. THOMPSON, M.Sc., *Department of Agriculture, The University, Leeds*

(With Plate 15)

There are many records of injury to grassland caused by caterpillars of the genus *Crambus* in Canada and the United States, where they are known commonly as 'sod webworms'. Such attacks however are not confined to grasses; cereal crops and tobacco frequently show marked injury following the ploughing of infested grassland. Attacks take place every year, but the degree of injury varies from season to season and in certain years attacks of abnormal severity occur: 1931 was reported from many parts of North America as being a particularly bad year for these pests and injury was widespread and severe. Noble (1932) states that twelve species of crambids are of economic importance in North America and all of these have similar habits. The relative prevalence of these species varies from season to season and in different parts of the country, and it appears to be usual in cases of severe grassland injury for more than one species to be involved in the attack.

Hitherto crambid moths, although extremely common, have not been regarded as of much economic significance in this country. During the autumn of 1941, however, a number of instances of injury to grassland in Yorkshire by caterpillars of these moths were brought to the notice of the writer. Larvae taken from these centres of attack were reared and, so far, all adults obtained belong to the species *Crambus hortuellus* Hb. This moth is one of the common species of economic importance in North America but is not regarded there as being one of the most harmful species. Injury was first reported at the beginning of October 1941. The areas affected were in the vicinity of Reeth in Swaledale and one isolated West Riding centre at Northowram, near Halifax. At the latter centre, severe injury was restricted to one field but there was evidence of lesser attack on adjacent fields.

Nature and extent of injury

Fields attacked were first visited on 17 Oct. by which time injury showed as large dead patches on the grassland, varying from several acres to as little as a quarter of an acre in extent. On these patches almost every plant had been eaten off at ground-level, the herbage lying dead upon the surface of the soil (Pl. 15, fig. 1). When the dead grass was moved aside numerous caterpillars* were exposed amongst the stem bases of the plants. Closer examination also revealed the presence of many silken cocoons, outwardly covered with soil particles and plant fibre (Pl. 15, fig. 2) which made them very difficult to see. These cocoons, like the caterpillars, were amongst the bases of the plants either upon the soil surface or lightly embedded in it; they contained caterpillars which at this date had not pupated. In some of the fields, attacked areas were very clearly defined, in others the injury was more general, though there were always patches more heavily attacked than the remainder of the field, the general effect being rather like a moth-eaten carpet.

* Mr H. M. Edelsten examined the caterpillars and kindly provided the following description: Colour translucent whitish ochreous, some with a greenish tinge. Head shining yellow brown covered with numerous setae. Prothoracic plate rather paler. There is also a pale anal plate. The body bears numerous setae. Spiracles small oval and black.

In Swaledale, the altitude of the area attacked was between 650–800 ft. and at Northowram 775 ft. above sea-level, and the fields were typical upland fields with a rather poor herbage. The soils were all acid in character with a *p*H between 3·44 and 5·14, nine out of twelve samples tested being under 4·5, and there was a considerable mat present. The areas attacked did not differ materially either in *p*H or herbage from adjacent unattacked areas and these factors appear to offer no explanation of the extremely local character of most of the attacks.

A botanical analysis of turfs from these fields, which were representative ot the general herbage in the districts was as follows: creeping red fescue and sheep's fescue, 30%; creeping bent, 25%; cocksfoot, 20%; Yorkshire fog, 15%; sweet vernal and perennial ryegrass, 5%; white clover, creeping buttercup and field woodrush, 5%. Almost all grasses were eaten but occasional strong-growing cocksfoot plants were uninjured and to a lesser extent this applied to Yorkshire fog also. White clover, buttercup and field woodrush appeared to be unharmed.

Degree of infestation

Most of the turf examinations were made after the time of cocoon formation. As the cocoons could be recovered and were considered to give some indication of the original degree of infestation, a series of turves were cut from a heavily infested field on 11 Nov.: six of these were trimmed to 6 in. squares and examined for cocoons with results as follows:

Turf	Cocoons apparently healthy	Cocoons bird damaged	Cocoons with fungus attack showing	Old cocoons	Total new cocoons
1	7	3	2	2	12
2	13	4	2	—	19
3	15	—	—	—	15
4	29	2	1	0	32
5	9	2	4	5	15
6	19	—	1	—	20
Totals	92	11	10	7	113

The numbers found indicate a population ranging from 48 to 128 new cocoons/sq. ft. This number, however, does not take into account caterpillars which failed to produce cocoons either because of disease or parasitism or because of the attacks of birds. All these factors are of importance, and it can safely be assumed that the original degree of infestation per sq. ft. was appreciably higher than this. Turf squares collected from Northowram cut on 11 Dec. gave similar figures. Three such squares yielded 15, 18 and 25 cocoons respectively or between 60 and 100/sq. ft. From this centre turves were also taken from areas adjoining those badly infested but not themselves showing caterpillar injury. Two of these showed 2 cocoons each, only one containing a healthy larva.

Stirrett & Arnott (1932) in Ontario record one extreme case of attack on a golf course where the infestation was 200 caterpillars/sq. ft., and state that turf injury will show following the presence of 12–15/sq. ft. Counts obtained by these writers were obtained by flooding unit areas with pyrethrum wash to bring the caterpillars to the surface.

Collections of cocoons from the remainder of the material from affected centres showed 198 cocoons apparently normal and twenty-three old cocoons which presumably had persisted from the previous year. The high number of old cocoons suggests that the heavy infestations in 1941 may have been built up over two or more years rather than in one season alone.

Life history

(*a*) *Laboratory observations*

On 17 Oct. 1941 thirty healthy larvae were collected near Reeth. Ten of these were placed in glass-topped boxes with a little soil and put in an incubator at 30° C. Seven formed cocoons between 22 and 27 Oct. and five moths emerged between 13 and 19 Nov., all belonging to the species *Crambus hortuellus* Hb. The period between commencement of cocoon formation and emergence of adults was between 17 and 29 days under these conditions. Adults on emergence did not leave the pupal shell completely within the cocoon but left it protruding slightly from the breach in the cocoon wall. Further attempts at rearing adults were made, batches of cocoons recovered from turf cut in affected fields being placed in the incubator between 14 Nov. and 12 Dec. 1941. As before, covered tins were used, the contents being kept moist. Adults emerged from these between 21 Dec. 1941 and 13 Jan. 1942. Unfortunately, much of the material became too dry and the emergence of adults was less than had been hoped for.

Many of the cocoons brought into the laboratory were examined and in every case contained larvae and not pupae. No pupae have been recovered from the fields attacked or from turf cut and kept out of doors by the end of January. It seems clear that the insect normally spends the winter as a caterpillar within the cocoon, pupation in the insects reared having been induced by incubation. A proportion of the incubated cocoons were opened at intervals and pupation under these conditions was found to take place about 15 days after incubation commenced; adults began to emerge 6 days later. It is hoped at a later date to obtain further information on the normal life history of this species, and to ascertain whether there is a spring feeding period prior to pupation, which appears to be usual in the case of crambids. For this purpose, infested turf material is being maintained under outdoor conditions.

Cocoons from which adults had been reared had all been picked out from the turf, and it was thought that other types of cocoons, possibly those of different species, might have been overlooked. Whole turfs were therefore incubated but from these also only *Crambus hortuellus* emerged.

(*b*) *Field observations*

Although not visited until mid-October reports showed that severe injury to the grassland first became evident during September when caterpillars were present in very large numbers. By 17 Oct. the great majority had formed cocoons. It is clear therefore that in the field the normal time of cocoon formation is the first 3 weeks in October, although as indicated already pupation within the cocoon does not take place at this stage.

It has not so far been possible to carry out observations over a sufficient length of time to obtain information on the complete life cycle, but Arnott (1934) made observations on moths caught at light traps which appear to indicate that *Crambus hortuellus* produces only one generation each year in North America, whereas some of the species of economic importance have two complete generations, and in others there is a partial third.

In North America several *Crambus* species are usually involved in attacks on grassland. Since various species of these moths also occur in this country, the same may be the case here. The number of moths so far reared is thirty, and although all these were *C. hortuellus*

the possibility of other species having been involved cannot be ruled out. *C. hortuellus* appears to differ from most other crambid moths in that silken galleries amongst the grass stems are absent. The cocoon is formed not in galleries but upon or embedded in the soil surface. No other types of cocoons have been noted so far in the turf examined.

Localization of attack

Noble (1932) associated the severity of attacks on lawns and golf courses in Ontario during 1930 and 1931 with the gradual concentration of the insects on artificially watered areas. These 2 years were abnormally dry and, on the watered areas, growth of the grasses was more luxuriant. Ainslie (1923) found attacks on meadow and pastureland to occur on the lower and damper parts of the fields where plant growth was more vigorous.

There appears to be little evidence of such an association in Yorkshire. Both 1940 and 1941 had unusually dry summers, but the areas attacked appeared to differ neither in herbage nor in soil moisture from adjacent areas free from attack. They were in fact in some cases on the higher parts of the fields and on pronounced slopes where natural drainage would tend to be better than the remainder. In some of the Swaledale fields, particularly, it is difficult to explain attacks on certain fields when surrounding fields with similar herbage and where soil moisture and general management were almost identical showed no injury.

Parasitism as a natural control factor

Relatively large numbers of the caterpillars found at the first examination were either dead or moribund. Some of these showed external evidence of fungus attack, apparently by *Isaria*. Other unhealthy caterpillars had symptoms possibly of bacterial origin, the body contents finally breaking down into a liquid mass. One of these, which was infested with eelworms, was submitted to Dr T. Goodey who reported that the eelworms present were not primary parasitic forms but free-living eelworms, larve of *Panagrolaimus rigidus*, which are widespread and common at the base of grass stems. In addition to caterpillars which did not form cocoons because of these factors many others became diseased after cocoon formation. From one batch of 198 cocoons examined forty-seven showed such attack. Other caterpillars were found to be parasitized by tachinids. Cocoons from two turves which were dissected and the caterpillars examined yielded one hymenopterous larva and many dipterous larvae. Rather more than one-third of the caterpillars had been parasitized. It is evident that parasitism plays an important part in the natural control of this species.

Birds

At one centre pheasants from adjoining woodland fed freely upon the caterpillars present. At most of the other centres, the flattened dead grass which had been killed by the caterpillars showed beak marks every few inches where starlings had been seeking out the larvae. These visits did not cease at cocoon formation as many of the cocoons showed evidence of having been torn open and the caterpillars removed.

Control

Many trials of control measures against crambid caterpillars are reported from North America (Stirrett & Arnott, 1932; Noble, 1932; Hutson, 1933; Stone & Elmore, 1937; Jewett, 1939). These experiments refer, however, only to attacks on lawns and golf courses

and no control measures are in general use in North America for treating pastures or meadow land. Where affected areas are to be ploughed a recommendation is made that ploughing should be done as early as possible before sowing to avoid the possibility of injury to cereal or other crops sown on such land.

In the attacks recorded in Yorkshire in 1941 the injury was completed by the time the writer's attention was called to them and the fields in question were not to be ploughed. The grass roots and stem bases appeared to be uninjured, and it was considered that a fair measure of natural recovery should be possible by the next spring. Turves which had been removed to the laboratory for examination indicated the possibility of this recovery and many plants developed new shoots. Suggestions were made, therefore, which had as their object the encouragement of the plants to re-establish themselves. Recommendations included the raking off and collection of the dead herbage from affected areas, the use of grass harrows to cut up the mat and, in view of the acid conditions of the fields, an immediate application of lime; these treatments to be followed by a phosphatic dressing in the spring. It seems probable that heavy harrowing to break up the surface would exercise some controlling effect upon the pest by disturbing the cocoons.

A point of interest in the comparison of the Yorkshire attacks with those recorded on lawns and golf courses in North America is that similar types of grasses are involved. Fescues and bent are typical lawn grasses, and factors on lawns which encourage these grasses such as a low *p*H and a low phosphatic figure are also present on the Yorkshire fields. It is thought that by raising the fertility of these fields and encouraging the better types of herbage grasses, which are largely absent at the present time, a type of herbage may be developed less liable to future attack.

In America it has been found that attacks on cereal and other crops are likely to develop following the ploughing out of infested grassland and along the margin of arable land adjoining infested grassland. In view of the present ploughing campaign in this country, it appears possible that attacks of a similar nature may develop here also, e.g. where early ploughing of infested fields is followed by the sowing of winter wheat. Cocoon formation, however, occurs here by early October, by which time little winter wheat has been sown and the risk of injury, therefore, would not appear to be great. Many American species have a spring feeding period before pupation occurs and should this prove to be the case here spring injury is a definite possibility.

Summary

Injury to grassland in Yorkshire by crambid caterpillars is described, the species involved being *Crambus hortuellus* Hb. Injury of this type, although common in North America, has not been met with previously in this country. Attacks were confined to upland fields in the North and West Ridings with a poor herbage in which fescues and bent predominated. Cocoons were counted as a means of indicating the degree of infestation which, in some of the turves examined, was as high as 128/sq. ft. Adults were reared in the laboratory by keeping caterpillars or cocoons in an incubator at 30° C. All adults so obtained belonged to the one species, which normally passes the winter in the caterpillar stage. The effect of parasitism and the attacks of birds are briefly discussed as natural control factors. Attacks by both occur before and after cocoon formation. Control measures adopted in North America on lawns and golf courses are not applicable to pasture or meadow land, and

measures recommended in Yorkshire aim at re-establishment of the turf and improvement of the herbage rather than direct control of the pest concerned.

The writer's thanks are due to Mr H. T. Jones for *p*H estimations of samples from infested fields, to Miss D. M. Turner for botanical analyses of turves collected, and to Mr J. C. F. Fryer for identification of the moths reared. Laboratory work in connexion with collection of caterpillars and cocoons and rearing of the moths was carried out by Mr D. Berryman to whom also thanks are due.

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EXPLANATION OF PLATE 15

Fig. 1. (*a*) undamaged turf; (*b*) turf damaged by caterpillars.
Fig. 2. Caterpillars, cocoons and adults.

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Fig. 1

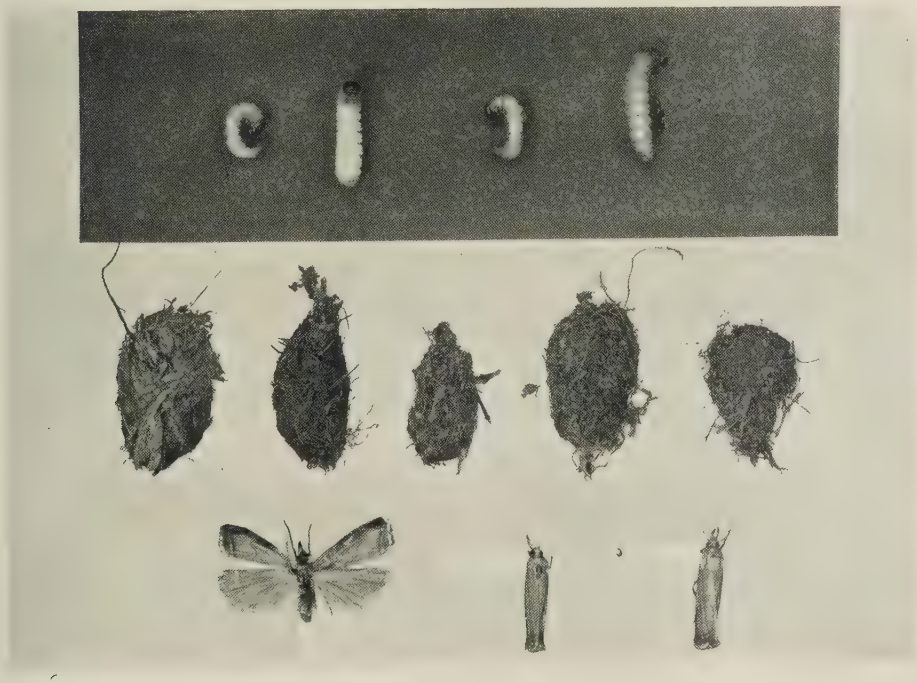

Fig. 2

LABORATORY TESTS OF BACTERICIDES ON THE PLUM AND CHERRY BACTERIAL CANKER ORGANISM (*PSEUDOMONAS MORS-PRUNORUM* WORMALD)

I. THE TOXICITY OF SOME INORGANIC MATERIALS, ESPECIALLY COPPER COMPOUNDS, AND THE EFFECT OF HYDROGEN ION CONCENTRATION ON THE ORGANISM

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The control of bacterial canker of plums and cherries now constitutes one of the major problems of the fruit grower. The only spray treatment (Wormald, 1932) is applications of Bordeaux mixture, but this has not proved consistently successful (Wormald, 1939, p. 68), and in wet weather may cause serious leaf spot and even premature defoliation. The purpose of the present work was to find out if copper, which had been used empirically, is the best available bactericide, and whether some other copper compound or preparation could with advantage replace Bordeaux mixture. The latter question led to a consideration of the effect of hydrogen-ion concentration on the organism. The work thus falls into three distinct, but closely related, sections.

Preliminary work by Wormald (1935) and by the present authors on *Pseudomonas mors-prunorum* (Worm.) included both bacteriostatic and bactericidal tests. In the former the substance to be tested was added to a standard nutrient solution, and the lowest concentration that prevented growth was determined. Some substances, notably copper compounds, reacted with the nutrient solution and invalidated the results. The experiments described in this paper are concerned only with bactericidal tests, the concentrations of toxic material required to kill the organisms in 15 min., 1 hr., and 2 hr. respectively being determined.

Experimental

Method

A pure culture of *P. mors-prunorum* isolated from plum canker in October, 1939, was grown for 2 days at 20° C. on nutrient agar (Difco) plus 5% sucrose and then suspended in sterile distilled water. No attempt was made to standardize the concentration of the bacteria beyond ensuring that a satisfactory turbidity was obtained each time. About 10 ml. of a sterile aqueous solution or suspension of the material to be tested was inoculated with 0·1 ml. of the bacterial suspension. After 15 min., 1 hr., and 2 hr., transfers were made by means of a sterile loop of 4 mm. diam. into tubes of nutrient broth plus 5% sucrose and these were incubated at 20° C. for at least 1 week. The resulting growth was noted periodically.

(1) *The metallic elements*

Most of the metals were tested as nitrates: in a few cases where the nitrate was not readily accessible the chloride was used. Thus, it was possible to test all the metals in solution, and differences that might have arisen from the effect of different acid radicles were avoided as far as possible. Data of the salts used are given in Table 1: (?) indicates doubt as to the precise amount of water of crystallization present in the molecule.

The salts were dissolved in water for testing, except vanadium chloride, which was brought into solution by treating with a little concentrated hydrochloric acid and neutralizing the solution by ammonia. For the preliminary survey solutions containing usually 0·1, 0·01, and 0·001 % of metal were tested, but for the more toxic metals gold, mercury, and silver, increased dilutions were used.

TABLE 1

Salt	Formula	Mol. wt.
Aluminium nitrate	$Al(NO_3)_3.9H_2O$	375·1
Barium nitrate	$Ba(NO_3)_2$	261·4
Beryllium nitrate	$Be(NO_3)_2.3H_2O$	187·1
Cadmium nitrate	$Cd(NO_3)_2.4H_2O$	308·5
Calcium nitrate	$Ca(NO_3)_2.4H_2O$	236·2
Cerium (ous) nitrate	$Ce(NO_3)_3.6H_2O$	434·3
Chromium nitrate	$Cr(NO_3)_3.9H_2O$	400·2
Cobalt (ous) nitrate	$Co(NO_3)_2.6H_2O$	291·1
Copper (ic) nitrate	$Cu(NO_3)_2.6H_2O$	295·7
Gold chloride (2 % w/v sol.)	$HAuCl_4.3H_2O$	394·0
Iron (ferric) nitrate	$Fe(NO_3)_3.9H_2O$	404·0
Lanthanum nitrate	$La(NO_3)_3.6H_2O$	433·0
Lead nitrate	$Pb(NO_3)_2$	331·2
Lithium nitrate	$LiNO_3$	69·0
Magnesium nitrate	$Mg(NO_3)_2.6H_2O$	256·4
Manganese (ous) nitrate	$Mn(NO_3)_2.6H_2O$	287·0
Mercury (ic) nitrate	$Hg(NO_3)_2.(?)2H_2O$	360·7
Mercury (ous) nitrate	$HgNO_3.(?)H_2O$	280·6
Nickel nitrate	$Ni(NO_3)_2.6H_2O$	290·8
Potassium nitrate	KNO_3	101·1
Silver nitrate	$AgNO_3$	169·9
Sodium nitrate	$NaNO_3$	85·0
Strontium nitrate	$Sr(NO_3)_2$	211·7
Thallium (ous) nitrate	$TlNO_3$	266·4
Thorium nitrate	$Th(NO_3)_4.4H_2O$	552·2
Uranium (uranyl) nitrate	$UO_2(NO_3)_2.6H_2O$	502·3
Vanadium (vanadyl) chloride	$VOCl_2$	137·9
Yttrium nitrate	$Y(NO_3)_3.6H_2O$	382·8
Zinc nitrate	$Zn(NO_3)_2.6H_2O$	297·5
Zirconium (zirconyl) nitrate	$ZrO(NO_3)_2.2H_2O$	266·9

Any solution whose hydrogen-ion concentration was not within the 'safe' range (cf. § 3) was excluded, e.g. bismuth, tin, and titanium. For the purpose of this survey variations in the results which might be due to *p*H differences were ignored. The results of the tests are expressed as the concentration of metal per cent. necessary to kill the organism in the time stated (Table 2). Barium, calcium, cerium, lanthanum, lead, lithium, magnesium, manganese, nickel, potassium, sodium, strontium, thallium, vanadium, and yttrium at 0·1 % concentration failed to kill the organism in 2 hr.

TABLE 2

Metal	15 min.	1 hr.	2 hr.
Zinc	n.t.*	n.t.	0·1
Aluminium	n.t.	0·1	0·1
Beryllium	0·1	0·1	0·1
Cadmium	0·1	0·1	0·1
Chromium	0·1	0·1	0·1
Thorium	0·1	0·1	0·1
Cobalt	0·1	0·1	0·01
Iron	0·1	0·1	0·01
Zirconium	0·1	0·1	0·01
Uranium	0·1	0·01	0·01
Copper	0·1	0·01	0·001
Gold	0·001	0·001	0·0001
Mercurous	0·0001	0·0001	0·00001
Silver	0·001	0·00001	0·00001
Mercuric	0·0001	0·00001	0·00001

* n.t. indicates not toxic at 0·1 % metal.

Metals toxic at a concentration of 0·1 % or lower, together with thallium and vanadium, were re-tested on a molecular and on a valency basis. The results (Table 3) are expressed as the molar (*M*) and the valency (*V*) concentrations per litre necessary to kill the organism in the time stated. Thus, for chromium nitrate ($Cr(NO_3)_3.9H_2O$), 0·01 *M* represents a concentration of 0·4002 %, and 0·01 *V* a concentration of 0·1334 %. In these dilute solutions *M* also represents the ionic concentration of the metals since none of these salts could give rise to more than one cation per molecule.

TABLE 3

Metal	15 min.		1 hr.		2 hr.	
	Molar (*M*)	Valency (*V*)	Molar (*M*)	Valency (*V*)	Molar (*M*)	Valency (*V*)
Aluminium	n.t.*	n.t.*	n.t.	n.t.	0·01	n.t.
Zinc	n.t.	n.t.	n.t.	n.t.	0·01	n.t.
Beryllium	n.t.	n.t.	0·01	n.t.	0·01	n.t.
Vanadium	n.t.	n.t.	0·01	n.t.	0·01	n.t.
Chromium	0·01	n.t.	0·01	n.t.	0·01	n.t.
Cobalt	0·01	n.t.	0·01	n.t.	0·01	n.t.
Iron	0·01	n.t.	0·01	n.t.	0·01	n.t.
Cadmium	0·01	n.t.	0·01	n.t.	0·01	0·01
Thorium	0·01	n.t.	0·01	0·01	0·01	0·01
Zirconium	0·01	n.t.	0·01	0·01	0·01	0·01
Thallium	0·01	0·01	0·01	0·01	0·01	0·01
Copper	0·01	0·01	0·001	0·01	0·0001	0·001
Uranium	0·01	0·01	0·001	0·01	0·0001	0·001
Gold	0·0001	0·001	0·0001	0·001	0·0001	0·0001
Mercurous	0·0001	0·0001	0·0001	0·0001	0·0001	0·0001
Silver	0·0001	0·0001	0·00001	0·00001	0·00001	0·00001
Mercuric	0·00001	0·0001	0·00001	0·00001	0·000001	0·00001

* n.t. indicates not toxic at 0·01 *M* or 0·01 *V*.

There is little in these results to suggest any periodic arrangement of toxicity except among the metals of the beryllium-mercury group where magnesium, zinc, beryllium, cadmium, and mercury show molar toxicities in approximately the same order as their atomic weights. Copper, uranium, gold, silver, and mercury stand out as the most toxic metals tested. The toxicity of mercury to micro-organisms is well known, and several workers have demonstrated the activity of silver. The results now presented are very broadly in agreement with a typical arrangement of the elements, e.g. that quoted by Topley & Wilson (1931, p. 106). There is close agreement in the positions of the most toxic metals, though uranium is now given a higher place. The chief divergences are the much lower positions of aluminium, cobalt, lead, nickel, and zinc in this investigation. McCallan & Wilcoxon (1934) found that silver was generally the most toxic of some forty elements tested against the spores of several moulds, and that uranium was on the whole not less toxic than copper. Contrary to the present results, however, cadmium, cerium, lead, and, to a less extent, chromium usually showed a considerable degree of activity. Copper is clearly the most toxic of the common base metals and, thus far, its use for the control of bacterial canker in orchard practice is justified.

The foregoing results show also that there are two types of concentration-time relation, viz.:

(*a*) among the less toxic elements, i.e. as far as thallium, where a minimum concentration, little affected by the period of exposure, is necessary for a complete kill.

(*b*) among the more toxic elements, i.e. from copper onwards, where the concentration of metal required to kill the organism decreases as the period of exposure increases.

The data in this communication are not adequate for a detailed examination of individual cases, but if this difference is substantiated by more critical experiments it may point to the mechanism of the action of these metallic bactericides.

(2) *Copper compounds and preparations*

To determine whether its toxicity is substantially influenced by the form in which the copper is applied, a variety of substances including soluble and insoluble copper compounds and several widely used spraying mixtures was tested. The copper compounds were cupric acetate, chlorate, chloride, nitrate, sulphate, and oxide, cuprous oxide, and cuprammonium sulphate ($Cu(NH_3)_4SO_4 . H_2O$). The solution of the last named was prepared from the dry solid and just sufficient ammonia was added to prevent precipitation on dilution. Bordeaux mixture was approximately '4 : 6 : 100' (0·3928% $CuSO_4 . 5H_2O$ and 0·6% $Ca(OH)_2$); for Burgundy mixture the lime was replaced by 0·425% crystalline sodium carbonate, and for Cheshunt compound by 2·2% ammonium carbonate. Each preparation was tested at 0·1, 0·01, and 0·001% copper (Table 4).

TABLE 4

Salt	*p*H at 0·1% Cu	15 min.	1 hr.	2 hr.
Cupric acetate	5·8	n.t.*	n.t.	0·001
Cupric chlorate	5·3	0·1	0·01	0·001
Cupric chloride	4·8	0·1	0·01	0·001
Cupric nitrate	4·8	n.t.	0·1	0·01
Cupric sulphate	5·0	n.t.	n.t.	0·01
Cuprammonium sulphate	9·2	n.t.	n.t.	0·01
Cheshunt compound	8·9	n.t.	0·1	0·01
Cuprous oxide	6·9	n.t.	n.t.	0·01
Cupric oxide	5·9	n.t.	n.t.	n.t.
Bordeaux mixture	*c.* 12·6	0·1	0·01	0·001
Burgundy mixture	6·5	n.t.	n.t.	0·1
Lime as in Bordeaux as $Ca(OH)_2$	*p*H at 0·6% $Ca(OH)_2$ *c.* 12·6	0·6	0·06	0·06

* n.t. indicates not toxic at 0·1% copper.

The toxicity of the soluble copper salts varies to an extent that cannot be explained by differences in hydrogen-ion concentration. Thus, the chloride and chlorate are considerably more toxic than the acetate, nitrate, sulphate, and cuprammonium sulphate. The 'sorting' technique of the present investigations is not, however, sufficiently discriminating to justify definite conclusions on this point. The toxicity of the insoluble copper compounds covers much the same range as that of the soluble salts, except that cupric oxide shows no activity at the highest concentration tested. Several authors have demonstrated the superior toxicity of cuprous oxide to cupric oxide against mould spores.

One of the most interesting features of these results is that Bordeaux mixture was only slightly more toxic than an equivalent concentration of calcium hydroxide alone. It thus appears that under the conditions of the test copper contributes to Bordeaux mixture very little toxic effect additional to that of the lime. Since it has been established that the calcium ion is not toxic, the effect appears to be due to the alkalinity of the medium. This was established when the hydroxides of calcium, barium, and sodium were compared with the corresponding chlorides. All the hydroxides were toxic in 1 hr. at concentrations of 0·06%, whereas the chlorides were not toxic even at much higher concentrations.

(3) *Hydrogen-ion concentration*

To fix approximately the alkaline limit of tolerance, a series of different sodium salts was tested, each at a concentration of 0·1 *M*. Although the series included salicylate and borate, the solutions of *p*H 9·3 or less were non-toxic and only those of *p*H 11·2 or more were toxic in 1 hr. Sodium hydroxide at 0·01 *M*, *p*H 12·0, was also toxic in 1 hr. (Table 5). The limiting *p*H was then fixed more closely by means of a range of buffer solutions consisting of mixtures of 0·2 *M* boric acid, 0·2 *M* potassium chloride, and 0·1 *M* sodium hydroxide giving hydrogen-ion concentrations of 9·4–10·6 in intervals of 0·2. A consistent end-point could not be obtained, but the organism was killed in 1 hr. at about *p*H 10·4. In a similar manner a selection of acid solutions, each containing 0·1% of acid, gave an end-point of about *p*H 3·0 (Table 6) for a complete kill in 1 hr.

TABLE 5

Substance	Conc. %=0·1 *M*	*p*H	Toxicity in 1 hr.
Sodium salicylate	1·60	6·3	Non-toxic
Sodium potassium tartrate	2·82	6·9	,,
Sodium ammonium phosphate	2·09	8·0	,,
Sodium bicarbonate	0·84	8·3	,,
Sodium phosphate ($Na_2HPO_4 . 12H_2O$)	3·58	9·2	,,
Sodium borate (borax)	3·82	9·3	,,
Sodium carbonate	1·06	11·2	Toxic
Sodium hydroxide	0·40	*c.* 12·3	,,
Sodium hydroxide	0·04 (0·01 *M*)	*c.* 12·0	,,

TABLE 6

Substance	*p*H	Toxicity in 1 hr.	Substance	*p*H	Toxicity in 1 hr.
Aminoacetic acid	7·0	Non-toxic	Sulphanilic acid	2·8	Toxic
Boric acid	5·6	,,	Formic acid	2·7	,,
Butyric acid	3·2	,,	Lactic acid	2·7	,,
Acetic acid	3·2	Toxic	Tartaric acid	2·7	,,
Phenylacetic acid	3·2	,,	Malonic acid	2·5	,,
Succinic acid	3·1	,,	Trichloracetic acid	2·3	,,
Salicylic acid	2·9	,,	Oxalic acid	2·2	,,
Citric acid	2·8	Non-toxic	Sulphuric acid	*c.* 1·8	,,
Malic acid	2·8	,,			

An attempt to fix the end-point by means of a potassium hydrogen phthalate-hydrochloric acid buffer showed that phthalic acid has a pronounced molecular toxicity to this organism apart from the hydrogen-ion concentration. Mixtures of 0·2 *M* disodium hydrogen phosphate and 0·1 *M* citric acid gave a suitable range, and by this means the limiting value was found to be about *p*H 3·2. There was some variation from experiment to experiment.

The limits of tolerance of *P. mors-prunorum* can, therefore, be stated as approximately *p*H 3·2–10·4. The organism does not survive 1 hr. exposure to a hydrogen-ion concentration outside this range. The somewhat anomalous limiting values shown in Table 6 may be related to the degree of molecular toxicity.

SUMMARY

In laboratory tests of twenty-nine metals in the form of soluble salts, mostly nitrates, the most toxic to *Pseudomonas mors-prunorum* (Worm.) were mercury, silver, gold, uranium, and copper, in descending order. Differences in toxicity among the various copper compounds and preparations could not be explained by differences in the hydrogen-ion concentration of the medium, but the outstanding activity of Bordeaux mixture among the insoluble forms of copper was accountable in terms of the alkalinity produced by the lime component. The range of tolerance of the organism to hydrogen-ion concentration was about *p*H 3·2 to 10·4.

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STUDIES ON THE MECHANISM OF FUNGICIDAL ACTION

IV. MERCURY

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I. Introduction

Compounds of mercury, particularly those containing organic radicals, are of increasing importance in the manufacture of seed dressings and for other fungicidal purposes. Little is known how the toxic agent exerts its effect, the most detailed study being that of Bodnár & Terényi (1932), on the chlamydospores of *Tilletia caries*. Mercurials have found little application as sprays or dusts in large-scale practice, on account of their high cost, and it is to these applications that the methods of investigation pursued in this work have particular relevance. Seed dressings are designed primarily against fungi present in the soil or on the seed coat, most of which reach the seedlings as actively growing hyphae rather than as spores. While there is a great physiological difference between hyphae and spores, the results presented in this paper may be of interest from the point of view of the biology of fungicidal action rather than of any particular application. In any case, the difficulties of quantitative work on the toxicology of fungal mycelia have been imperfectly surmounted.

The method adopted was to apply the theory of variability (Parker-Rhodes, 1942) to dosage-mortality figures obtained for two fungi and one bacterium with a representative range of mercury compounds, with a view to obtaining evidence as to what types of compounds could be absorbed by the spores. In particular, the mode of action of alkyl-mercury compounds was considered of special interest.

II. Experimental technique

Biological materials

The fungi used were *Macrosporium sarcinaeforme* and *Botrytis allii*, both direct descendants of those used in earlier work (Parker-Rhodes, 1941); the methods of culture were the same throughout. The *Macrosporium* culture was derived from one of a series of monospore isolations from the stock; different isolates differed considerably among themselves in the morphological and physiological characteristics of their germination, some being definitely abnormal. The strain for the present work was chosen as having normal germ tubes, and a sensitivity to copper sulphate as nearly as possible the same as that of the original strain. In view of the wide variability of monospore isolates it is clearly possible for a strain of this fungus, perpetuated by multispore subculturing, to alter progressively by a process of cultural selection, for at each subculturing there will be a tendency for the mycelia produced by those spores best able to thrive, on the particular medium used, to make a greater contribution to the spores used for the next subculture. Thus, if one batch of medium is accidentally contaminated with a subtoxic concentration of copper, one may expect that the spores obtained from cultures on such a medium after a few generations will be more resistant to the toxic action of copper than those obtained from an uncontaminated medium. When it is necessary to use a medium, such as malt-extract agar, containing ingredients which are not accurately standardized, it is probably advisable to resort to monospore isolations from time to time in order to maintain the constancy of the strain. The strain of *Macrosporium* used in this laboratory had altered considerably in its sensitivity to copper in the course of a year, though how far the change was due to such causes as these it is impossible to say.

The bacterium was *Bacillus agri*, derived from a culture obtained from the National Collection of Type Cultures in 1940. The stock cultures were maintained at about 21° C. on beef-extract peptone agar, and renewed about once a fortnight.

Chemical materials

The following compounds of mercury were employed: mercuric acetate, mercuric chloride, methylmercuric nitrate (CH_3HgNO_3), tolylmercuric acetate ($CH_3C_6H_4Hg.OOCCH_3$), and mercurous chloride. All were used in the form of laboratory reagents. In the concentrations required in this work all compounds except the last could be used in solution, but mercurous chloride had to be applied as a spray; the sample used was from the Leyton Manufacturing Co., Ltd., guaranteed to conform to B.P. specification.

Experimental methods

The methods used for the fungi were essentially the same as those described previously (Parker-Rhodes, 1941), except in two particulars: (*a*) the criterion of germination of spores of *Botrytis allii* was taken to be the presence of one or more pairs of points of inversion in the optical outline of the spore, which was found easier to apply at a glance and to give more consistent results than that previously adopted; and (*b*) in many tests twelve different concentrations rather than eight were used to cover the critical range, which was found to enhance considerably the amount of information obtainable from each test and thus save unnecessary repetitions.

The technique devised for applying the theory of variability to bactericidal action was as follows: 22 hr. before the commencement of an experiment a tube containing 10 c.c. of half-strength beef-extract peptone liquid medium was inoculated from a stock culture of the bacterium, and incubated overnight at 25° C. The following day a series of dilutions of the solution to be tested were prepared in test-tubes, together with distilled water as a control; 1 c.c. of each dilution was taken. Meanwhile, a corresponding number of tubes of agar containing half-strength beef-extract peptone medium, had been prepared containing 5 c.c. each (the agar was measured out hot with a 5 c.c. pipette), and autoclaved; these were transferred to a water-bath maintained at about 70° C. One c.c. of the liquid culture of bacteria was then transferred by a pipette to the first test-tube containing the toxic solution, and the time noted to the nearest second. One minute later a second c.c. was transferred to the second tube, and so on. Aseptic precautions were not observed during these operations, on the grounds that (*a*) the number of contaminating bacteria introduced is negligible in comparison with those already present, and (*b*) in counting the plates, contaminatory bacteria can be distinguished by the appearance of their colonies. After 7 min. all the eight portions of test solutions were diluted with an equal volume of bacterial suspension: $4\frac{1}{2}$ min. after the last treatment a tube of agar was withdrawn from the water-bath to cool, and further tubes were taken out at intervals of 1 min. thereafter, these tubes being arranged in the order of their withdrawal. Half a minute after the fourth tube was removed, 24 cu. mm. of the mixture in the first treatment tube was measured out and introduced into the first tube of agar by means of a micropipette; to ensure proper mixing air was bubbled through from the pipette after discharge of the bacteria, and the inoculated tube was briskly rotated between the hands before pouring. The tubes were poured into sterilized Petri dishes; the inoculation of tubes proceeded at intervals of 1 min. until all the eight tubes had been poured. The plates were then stacked and incubated for 44 hr. at 25° C.; each contained a uniform suspension of bacteria in agar, which had been subjected to a known concentration of a given toxic agent for 15 min., together with a negligible concentration of the same toxin carried over with the 24 cu. mm. of inoculum. After incubation the bacteria formed colonies large enough to be counted under the microscope. Counting was carried out using a $\frac{2}{3}$ in. objective, and either a 10× or 6× eyepiece; the eyepiece was fitted with a diphragm in the form of a sector of the field, of angle 47°, together with a small circle in the centre. A field was selected at random, viewing the plate through its underside, and the thickness of the agar measured by means of the fine adjustment. The number of colonies in the field was then counted by placing the sectorial diaphragm in its eight successive positions (allowing a small overlap each time); the central circle in the diaphragm avoids the possibility of missing a colony exactly in the centre of the field. Without the diaphragm it is impossible to concentrate sufficiently to count accurately fields with more than about forty colonies. Five fields were counted in each plate, and from these were obtained five independent estimates of the concentrations of viable bacteria in the agar. By comparison with the controls, the proportional mortality can be calculated. The calculation of statistics from this point was carried out as in the case of spore germination counts; each field counted, being a statistically independent estimate, contributed 1 to the weight attached to the

estimate made of the mortality; but in order to allow for differences in the volume of agar counted in different cases, the ratio of the sum of the thicknesses of agar in all five fields counted in each treatment to the corresponding sum in the control plate was used as a further coefficient in calculating the weights.

III. Presentation of results

The results obtained are tabulated in Tables 1–14. In these tables, x is the concentration applied (in arbitrary units), q the proportional mortality of the spores or cells, and n' the weighting factor,* which in the case of the fungi represents the number of drops counted, and with the bacteria, five times the coefficient mentioned at the end of the previous section. Mercurous chloride was used both in the form of a freshly made up suspension, and after standing for 3 days; unfortunately, the technique adopted for investigating the bacteria could not be adapted to the study of insoluble compounds, so that this substance was omitted from these experiments. From the results thus tabulated, the following statistics were calculated: U, the variability; $M\,(=1/A)$ used in assessing the significance of differences in U, by means of the formula $(U_1+U_2-2)\sqrt{(U_1U_2)/(M_1+M_2)}\,U_1U_2=3{\cdot}84$ (for significance at the 5 % level) and, thirdly, the 'LD 50'. The latter was calculated from the absolute values of x (not given in Tables 1–14) in terms of p.p.m. Hg. As noticed elsewhere in this work, considerable fluctuations were observed in this quantity, the greatest value being up to ×6 the least observed for a single compound. The values obtained with *Bacillus agri* were much more consistent. The index of variation was taken as zero with all three organisms.

Effect of mercury compounds on Macrosporium

Table 1. *Mercuric acetate*

log x	q	n'
0·30	0·000	5
0·32	0·020	5
0·40	0·089	5
0·70	0·051	5
0·72	0·045	5
0·81	0·544	5
1·11	0·933	5
1·13	0·955	5
1·21	0·766	5
1·52	0·973	5
1·54	0·979	5
1·62	0·872	4
1·92	0·9910	5
2·02	0·9830	5
2·32	1·0000	5

Table 2. *Mercuric chloride*

log x	q	n'
0·00	0·024	5
0·20	0·009	5
0·40	0·132	3
0·49	0·117	5
0·77	0·425	5
0·81	0·213	3
1·05	0·697	5
1·21	0·691	5
1·35	0·9955	5
1·62	0·9919	5

Table 3. *Methylmercuric nitrate*

log x	q	n'
0·00	0·016	5
0·35	0·037	5
0·40	0·118	5
0·50	0·268	5
0·81	0·641	5
0·91	0·766	5
1·04	0·955	5
1·31	0·964	5
1·72	0·980	5
2·12	0·9960	5
2·53	1·0000	5

Table 4. *Tolylmercuric acetate*

log x	q	n'
0·00	0·000	5
0·81	0·064	5
1·21	0·201	5
1·62	0·859	5
2·02	0·944	5
2·43	0·9960	5
2·83	1·0000	5

Table 5. *Mercurous chloride (fresh)*

log x	q	n'
0·69	0·064	5
1·39	0·133	6
2·08	0·435	6
2·77	0·563	6
3·46	0·868	6
4·16	0·862	6
4·85	0·962	6
5·54	0·9927	6
6·23	1·0000	6

Table 6. *Mercurous chloride (3 days old)*

log x	q	n'
0·00	0·050	6
0·69	0·075	5
1·39	0·236	6
1·80	0·523	6
2·08	0·608	6
2·49	0·683	6
2·77	0·913	6
3·19	0·9859	6
3·46	0·9800	6
3·88	0·9968	6

* In calculating the statistics corrections were applied to n' in accordance with the note at the end of this paper.

Effect of mercury compounds on Botrytis

TABLE 7. *Mercuric acetate*

log x	q	n'	log x	q	n'	log x	q	n'
0·00	0·014	5	1·61	0·294	5	2·77	0·560	5
0·40	0·086	5	1·80	0·478	5	2·83	0·768	4
0·69	0·137	4	2·02	0·419	5	3·19	0·827	4
0·80	0·333	4	2·08	0·636	5	3·23	0·942	5
1·21	0·485	5	2·42	0·590	5	3·57	0·934	5
1·39	0·394	5	2·49	0·643	5	3·64	0·9889	5
						4·26	1·0000	5

TABLE 8. *Mercuric chloride*

log x	q	n'
0·00	0·163	5
0·69	0·321	5
1·39	0·439	5
2·08	0·679	5
2·78	0·860	5
3·47	0·801	5
4·16	0·801	4

TABLE 9. *Methylmercuric nitrate*

log x	q	n'
0·00	0·166	5
0·69	0·156	4
1·39	0·183	5
2·08	0·337	5
2·78	0·505	5
3·47	0·461	4
4·16	0·805	5
4·85	0·721	4

TABLE 10. *Mercurous chloride (fresh)*

log x	q	n'
0·00	0·286	6
0·69	0·439	5
1·39	0·562	6
2·08	0·676	5
2·78	0·748	5
4·16	0·918	6
5·54	0·958	6
6·92	0·9810	6

Effect of mercury compounds on Bacillus agri

TABLE 11. *Mercuric acetate*

log x	q	n'
0·00	0·084	4·55
0·69	0·217	5·20
1·39	0·503	5·45
2·08	0·561	5·70
2·78	0·731	4·90
3·47	0·655	3·95
0·69	0·223	3·75
1·39	0·391	4·30
3·47	0·823	4·80
4·16	0·955	6·20
0·00	0·092	3·50
0·69	0·456	3·50
1·39	0·517	3·45
2·08	0·830	6·55
2·78	0·9675	4·35
3·47	0·9672	3·90
4·16	0·9766	5·20

TABLE 12. *Mercuric chloride*

log x	q	n'
0·00	0·000	5·00
0·40	0·008	6·25
0·81	0·108	5·05
1·21	0·420	5·85
2·02	0·941	10·85
2·43	0·9624	11·80
0·35	0·028	6·25
1·04	0·258	5·25
1·74	0·872	15·15
2·43	0·9990	9·45
3·13	0·9995	14·85
3·82	0·9983	17·15
0·35	0·038	4·35
1·04	0·391	7·95
1·74	0·396	8·30
2·43	0·908	12·15
3·13	0·9875	11·65
3·82	1·0000	8·00

TABLE 13. *Methylmercuric nitrate*

log x	q	n'
0·00	0·000	5·00
0·69	0·016	6·15
1·39	0·057	5·70
2·08	0·107	4·75
2·78	0·092	4·85
3·47	0·140	4·40
1·39	0·000	2·00
2·08	0·195	5·20
2·78	0·253	5·40
3·47	0·164	4·15
4·16	0·502	5·00
4·86	0·899	5·75

TABLE 14. *Tolylmercuric acetate*

log x	q	n'
0·00	0·000	5·00
0·69	0·116	4·45
1·39	0·604	5·40
2·08	0·617	4·40
2·78	0·739	5·20
3·47	0·880	5·30
4·16	0·804	4·35

TABLE 15. *Summary of results*

	Statistics								
	Macrosporium			*Botrytis*			*Bacillus agri*		
Compound tested	LD 50*	*M*	*U*	LD 50*	*M*	*U*	LD 50*	*M*	*U*
A. Mercuric acetate	6·9	0·164	0·154	1·54	0·012	1·49	0·39	0·015	1·28
B. Mercuric chloride	21·4	0·325	0·107	0·104	0·033	1·69	0·65	0·055	0·33
C. Methylmercuric nitrate	0·014	0·486	0·066	0·044	0·009	5·56	0·16	0·026	2·44
D. Tolylmercuric acetate	3·4	0·670	0·098				0·67	0·023	1·32
E. Mercurous chloride (stale)	—	0·041	0·498						
F. Mercurous chloride (fresh)	—	0·024	1·38	0·057†	0·006	5·78			
Combined figures:									
A and B				—	0·009	1·54			
A, C and D							—	0·001	1·50
A, B, C and D	—	0·069	0·118						
C and F				—	0·004	5·69			

* In p.p.m. Hg.

† This figure was calculated assuming complete dissolution of the solid in the medium.

Significant differences (see Table 15)

(*a*) *Macrosporium sarcinaeforme*. No significant difference among the variabilities to A, B, C and D; to E the variability is greater than that to A, B, C and D, but not more than $\times 4$; to F it is greater than $4\times$ that to A, B, C and D, but not more than $\times 9$. The difference in *U* between E and F is not significant.

(*b*) *Botrytis allii*. No significant difference between the variabilities to A and B, nor those to C and F; the variability to A and B is less than that to C and F, but not less than $\frac{1}{4}$ or greater than $\frac{1}{9}$ of it.

(*c*) *Bacillus agri*. The variabilities to A, C and D are each greater than that to B, but neither separately nor combined are they greater than $4\times$ this.

Note. In Tables 1–10, where two independent experiments have been performed on one compound, the figures have been combined, and the statistics calculated as from the tables; but with the bacteria (Tables 11–14) the experiments were usually repeated with identical concentrations, and the several repetitions are recorded separately; in this case the statistics were calculated separately for each experiment, and combined, with their appropriate weights, afterwards.

IV. DISCUSSION

Absorption of mercury by Macrosporium sarcinaeforme. From the similarity of the figures for the variability to mercuric acetate, mercuric chloride, and the organic complexes, it is probable that all can be absorbed equally, both as ions and as neutral molecules, or that all compounds are reduced to an absorbable form by a similar mechanism. As regards the inorganic compounds, the reduction of the salts to metallic mercury by the spore secretion* might be such a mechanism, but this is unlikely to apply to the very stable CH_3Hg^+ and $CH_3C_6H_4Hg^+$ ions and furthermore leads to the conclusion that the variability to mercurous chloride, which yields mercury spontaneously by the reaction $Hg_2Cl_2 \rightarrow Hg + HgCl_2$, should be less than that to the mercuric salts which require the intervention of the spores to produce the metal. Actually, the variability to the latter compound in a freshly prepared

* For this suggestion I am indebted to Mr J. R. Booer.

spray is more than 4× that to the mercuric compounds. From the spontaneous decomposition of mercurous chloride to yield mercuric chloride, one would expect the ratio to be not more than four, but for the fact that the mercuric chloride will inevitably be reduced to some extent by the spore secretion to yield mercury, thereby retarding the initial decomposition through the presence of a common product. In this case the theory of variability predicts an increase in the variability over the normal value, though whether the difference is noticeable depends entirely on the extent to which the secondary reduction takes place. The figure for 3 days old suspension is intermediate, presumably on account of the presence of a greater quantity of mercuric chloride.

It is concluded that the neutral molecule $HgCl_2$ and the ions Hg^{++}, CH_3Hg^+, and $CH_3C_6H_4Hg^+$ can all be absorbed, but not the molecule Hg_2Cl_2.

Absorption of mercury by Botrytis allii. In this case we find that whereas the mercuric ion of the acetate can be absorbed, and also the little-dissociated mercuric chloride, the methylmercuric ion resists absorption. The figures are compatible with the view that some constituent of the spore secretion decomposes the complex to yield simple Hg^{++} ions, which are responsible for the toxicity of this compound. The variability of the spores to mercurous chloride is almost the same as that to the organic complex; this result does not dispose of the hypothesis that $HgCl_2$ formed by decomposition is effective, but by the argument given above a larger figure might have been expected. Here also, however, the figures are in contradiction to the view that metallic mercury is the effective agent.

Absorption of mercury by Bacillus agri. No evidence was found of a difference in the variability of the bacteria in their tolerance to mercuric and tolylmercuric acetate and to methylmercuric nitrate; these compounds resemble one another, and differ from mercuric chloride, in providing a considerable concentration of mercurial cations in aqueous solution. If, however, the cells could be affected by *any* neutral mercurial molecule, theory predicts that their variability to mercuric chloride would be ×4 greater than that to the dissociated compounds. This is so far in contradiction to the facts as to suggest that the chloride is peculiar in its permeativity, and that the other compounds must be built up into halide molecules, or some other complex, under conditions prevailing in the immediate vicinity of each bacterial cell, before the toxic element can be absorbed. But it is necessary to interpose a caution to this interpretation, since in the experimental procedure the toxin had of necessity to operate in a medium far from chemically neutral, and it is possible that the more dissociated compounds were rendered unavailable to the bacteria by some reaction with a constituent of the culture medium.

Interpretation of the results. The interpretation to be put upon these results depends on what view is adopted concerning the role of the mercury in the metabolism of the spores or cells. Either it enters irreversibly into the protoplasm, or it is merely adsorbed onto the outer membrane, achieving only a reversible inactivation. The latter phenomenon was observed by Bodnár & Terényi (1932) in a study of the action of mercury on the chlamydospores of *Tilletia caries*; their results indicated that this fungus could *ad*sorb mercuric ions, but could only *ab*sorb lipophilic compounds such as the mercuric halides. The latter produced an irreversible inactivation (i.e. death) of the spores, whereas such salts as the acetate, from which ions were adsorbed, produced only a suspension of germination, reversible by means of suitable eluents. Mercuric cyanide, providing no ions at all, could not achieve the necessary first stage of adsorption and was thus non-toxic. The conidia

of *Macrosporium* spp. have a thickened and pigmented spore-wall, which may well have similar physical properties to that of the chlamydospores of *Tilletia* (Bodnár & Terényi state that sawdust and talc powder exhibited similar adsorptive properties). Furthermore, if adsorbed toxins are capable of causing a reversible inactivation of the spores, the distribution of susceptibility of the spores to such an effect should obey similar laws to that of any other type of toxication (though we may expect the magnitude of the variability to be less). The word 'absorb' as used above must therefore be so interpreted as to include adsorption as well as actual permeation; as to which possibility occurs in the present case, nothing can be said on the basis of these investigations, but the possibility must be borne in mind that the two fungi differ in this respect, so that we are comparing essentially dissimilar phenomena. As to the bacteria, the distinction among antiseptics between bactericidal and bacteriostatic action is well known; but the procedure adopted makes it very unlikely that what is here observed is anything but a true bactericidal action of the mercury.

It is of some interest that both the acetate and the chloride give the same variability with the two fungi. The dissociation constants of mercuric chloride are given by Morse (1901) as: first stage $2{\cdot}5 \times 10^{-7}$, second stage $1{\cdot}0 \times 10^{-14}$; whereas the acetate is much dissociated in dilute solution. There is, therefore, no doubt but that both ions and neutral molecules containing mercury behave alike, whether by adsorption or by permeation, to both *Macrosporium* and *Botrytis*. It is also noteworthy that the methylmercuric ion can affect *Macrosporium* directly, but not *Botrytis*; if in both cases true permeation is involved this is in agreement with the more lipoidal character of the spore wall of the former.

The chief remaining observation of interest is the much greater toxicity of methylmercuric nitrate to the fungi than any of the other compounds; the effect was less marked in the case of *Botrytis*, while for the bacteria it can only be said that this was the most toxic compound of those tested. In view of the close structural affinity between the cationic portions of the molecules, a test was made of infusible white precipitate (amidomercuric chloride) on *Macrosporium*. It was found that the toxicity was of the same order as that of other compounds; the variability was about 0·1.

Summary

Figures are tabulated showing the effect of a range of compounds of mercury on conidia of two species of ascomycetes, and one of bacteria. A technique for testing the action of toxins on bacteria in such a form as to enable them to be analysed by the methods of the theory of variability is described. From the estimates of the variability of the spores and cells to the various toxins used it is inferred that: (*a*) *Macrosporium sarcinaeforme* can absorb all the mercuric compounds tested, but requires the molecule of mercurous chloride to be dissociated to the metal and the mercuric salt before it can be absorbed; (*b*) *Botrytis allii* differs in being unable to absorb the methylmercuric ion in its native state; and (*c*) *Bacillus agri* cannot absorb mercury except in the form of its chloride, and possibly other combined forms which are produced under the influence of the diffusate from the cells. The significance to be attached to the term 'absorption' in connexion with the fungicidal action of mercury is discussed. Evidence is adduced that in these experiments the toxic effects against bacteria were bactericidal rather than bacteriostatic in nature. The superior toxicity of methylmercuric nitrate over the other compounds used is commented upon.

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NOTE

The quantitative estimation of the mutual dependence of spores used in fungicidal germination tests

In computing the results of fungicidal tests based on germination counts by the method of probit analysis, it is necessary to assign to each point on the probit regression line a weight, dependent on the number of spores on which it is based, and on its position on the curve. If every spore were statistically entirely independent of its neighbours the weight of the point should be taken as $N\frac{z^2}{pq}$, where N is the number of spores counted, z the ordinate of the normal curve $z = e^{-\frac{1}{2}y^2}/\sqrt{(2\pi)}$ in which y represents the expected probit mortality, and p and q are the proportions of the spores expected to germinate and to fail to germinate respectively. But in fact, owing to the mutual influence of the spores in one drop on each other, they cannot act in a strictly independent manner, so that N must be taken to represent not the total number of spores counted, but a smaller number.

Hitherto in the writer's work it has been assumed that all the spores in one drop are mutually dependent, so that N was put equal to the number of drops from which counts were made. It has now become apparent that this assumption is false. If the value of χ^2, representing departure of the observed points from linearity, is compared in any given case with the mean value expected with the given number of degrees of freedom, it is generally found to fall below it; on summing *all* the χ^2-values which have hitherto been obtained with each species of fungus used, and comparing this $\Sigma(\chi^2)$ with the expected value, which is $\Sigma(n)$, a very considerable deviation is found, which would imply, if the assumption with regard to N were true, that the results were much more closely in accordance with expectation, than would be expected by random sampling. This can be explained either by assuming a subconscious bias on the part of the experimenter; or, which is more probable, by supposing that the spores are *less mutually dependent* than has been assumed, since the estimate of χ^2 is directly proportional to N. If we postulate that from a total count of some 30,000 spores the aggregate observed χ^2 should not depart significantly from expectation, the ratio of the value estimated using the false assumption to that expected gives a numerical measure of the error involved. The figures are as follows:

		$\Sigma(\chi^2)$			
	$\Sigma(n)$	Calc.	Obs.	Ratio	D
Macrosporium sarcinaeforme	137	75·19	136·50	1·82	27
Botrytis allii	66	23·00	65·50	2·84	18
Bacillus agri (assuming complete independence)	17	874·8	16·34	0·0185	54

D is the number of spores (hitherto assumed to be 50) or cells which for statistical purposes may be assumed to form a mutually dependent group. It will clearly depend on the experimental technique adopted.

This correction affects not only χ^2 but also the statistic A used in estimating the significance of differences in variability, the effect being to enhance the significance of any difference. On applying this correction to the writer's previous work it is found that no change in interpretation is necessitated, save that in the first paper (*Ann. appl. Biol.* **28**, 389) the variability of *Macrosporium* to cupric glycinate now appears significantly greater than to any other cupric complex; and that in the third paper (on sulphur compounds) there is now one more significant difference in the values of $\alpha^2 U_\alpha$ namely that the value for sodium dithionate is significantly lower than that for sodium hydrogen sulphite (acid solution); this supports the idea mentioned in the discussion that the pyrosulphite ion cannot be directly absorbed by *Macrosporium* spores.

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STUDIES UPON THE COPPER FUNGICIDES

V. A CRITICAL EXAMINATION OF THE FUNGICIDAL VALUE OF COPPER COMPOUNDS

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(With 2 Text-figures)

The occurrence of fungicidal properties throughout a wide range of copper compounds was shown (Marsh *et al.* 1937) by field trials in which relative efficiency was assessed by the degree of control of potato blight (*Phytophthora infestans*). The result of the field trial is, however, an integration of the many factors determining protective value (see Horsfall *et al.* 1937) and, for the more detailed investigation now described, the fungicidal values were examined under laboratory conditions in which factors associated with retention and tenacity could be held constant and those associated with host plant interaction could be eliminated. Moreover, in the field trial it is necessary to add to the spray accessory substances to serve as dispersing agents and spreaders, which might complicate the interpretation of fungicidal value of their interaction with or effect on the reaction of the spore to the copper compound under investigation. The purpose of the present work is to examine the reaction of the spore to the copper compound under conditions free, as far as practicable, from interference by nutrient or impurity and to apply the results obtained to the examination of hypotheses of the mode of toxic action. The compounds examined were selected primarily from the latter point of view, as parallel investigations of the chemistry of spore excretions and of availability were in progress.

Experimental

Methods

The biological assays were mostly carried out by the in vitro slide method (Marsh, 1938). To ensure standard wetting properties, the slides were cellulosed by immersion in 2·5 % cellulose nitrate in butyl acetate, the solvent being allowed to evaporate. The spray to be tested was applied, at 2 atm. pressure, by means of an atomizer jet placed 24 in. from the slide which was exposed for 10 sec., the atomizer being adjusted to yield an evenly dispersed deposit of 0·0393 g. spray/sq. in. of slide, an amount insufficient to cause run-off. The sprayed slides, protected from dust, were dried at laboratory temperature. Each slide was then sown, by means of a micropipette, with three separate drops of spore suspension, each drop being 0·04 ml. spreading on the slide to 0·7 cm. diam. and containing 250–300 spores. The slides were placed on glass racks in moist chambers and incubated at 20–23·5° C. for approximately 48 hr. The percentage germination in each drop was then determined from a count of 100 spores. Immature spores of *Macrosporium sarcinaeforme*, distinguished by their lighter colour, and spores with attached conidiophores were disregarded together with those at the periphery of the droplet. With these precautions no difficulty was met in judging germination which seldom differed in character throughout the concentration range. On a few slides, 27 in over 1100, abnormal germination occurred in the form of numerous pimples of length not greater than their diameter protruding from the spore wall. The counts from these slides were ignored. With spores of *Venturia inaequalis* and *V. pirina*, the development of the germ tube was comparatively easy to observe, the spore contents becoming lighter in colour and points of inflexion appearing in the spore outline.

The range of concentrations over which each compound was tested was determined by the expected toxicity but, in all cases, the dosages within the range were in geometric progression with ratio 2

or $\sqrt{2}$, ratios also found satisfactory by McCallan *et al.* (1941). Each compound to be tested was made up in water to the required strength, stability in the resultant suspension being secured by grinding and maintained during spraying by a mechanical stirrer.

The spore suspensions employed were, principally, of *Macrosporium sarcinaeforme*, derived originally from a culture brought from Ithaca, N.Y., by Prof. J. G. Horsfall. These were obtained by flooding the slope of a 20–25-day-old culture on malt-extract agar with sterile water. The surface of the culture was gently stroked with a blunt-ended glass rod to promote wetting and, after inverting the tube 8–10 times, the spore suspension was transferred to a small centrifuge cup. The spores were then spun off and washed thrice with sterile water to remove nutrient dissolved from the culture, a precaution not taken in some of the earlier tests. The spore suspension was then diluted to the required density with sterile water. To obtain spore suspensions of *Venturia inaequalis* and *V. pirina*, portions of leaves bearing young conidial infections were shaken with sterile water but, to avoid the detachment of immature spores, the glass-rod treatment was omitted. The resultant suspension was washed thrice at the centrifuge and diluted to the appropriate density.

An alternative technique, referred to as the method on unsprayed slides, was sometimes employed with water-soluble copper derivatives. Solutions at double the concentration to be tested were mixed with equal volumes of spore suspension and the germination, in 0·04 ml. drops on unsprayed cellulosed slides, observed as in the drops on the sprayed slides.

Allowance for day-to-day variation in spore resistance was made by taking samples from the same spore suspension for each series of tests, and a common fungicide, either Bordeaux mixture or copper sulphate, was included as a check in each series.

Compounds tested

General methods of preparation. For soluble salts, a solution of the acid was shaken continuously for 24 hr. with an excess of pure cupric oxide. The filtrate was evaporated down until the copper salt crystallized out. For insoluble salts, the equivalent of the acid was determined by titration so that its solution could be exactly neutralized with carbonate-free sodium hydroxide. The solution of the sodium salt thus obtained was treated with a slight excess of cupric sulphate solution; the precipitated salt, after at least three washings by decantation, was filtered off, washed free from sulphate and dried either at 110° C. or in the air.

The following cupric derivatives were prepared by these methods and their copper content determined by the iodometric method:

Adipate, 31·31% Cu; $C_6H_8O_4Cu$ requires 30·62% Cu.
Alaninate, 24·78% Cu, 10·93% N; $C_6H_{12}O_4N_2Cu,H_2O$ requires 24·67% Cu, 10·87% N.
Benzoate, 18·11% Cu; $C_{14}H_{10}O_4Cu,2H_2O$ requires 18·61% Cu.
Dinitro-*o*-cresylate, 12·06% Cu, 10·60% N; $(C_7H_5O_5N_2)_2Cu,4H_2O$ requires 12·00% Cu, 10·57% N.
Glycinate, 27·65% Cu, 12·18% N; $C_4H_8O_4N_2Cu,H_2O$ requires 27·74% Cu, 12·23% N.
Hippurate, 15·35% Cu; $C_{18}H_{16}O_6N_2Cu$ requires 15·16% Cu.
Lactate, 23·45% Cu; $C_6H_{10}O_6Cu,2H_2O$ requires 22·90% Cu.
Malonate, 29·27% Cu; $C_3H_2O_4Cu,3H_2O$ requires 28·96% Cu.
Mucate, 19·28% Cu; $C_6H_8O_8Cu,4H_2O$ requires 18·49% Cu.
Oxalate, 39·60% Cu; $C_2O_4Cu,\frac{1}{2}H_2O$ requires 39·59% Cu.
Phosphate, 45·36% Cu; $Cu_3(PO_4)_2,3H_2O$ requires 43·86% Cu.
Phthalate, 27·58% Cu; $C_8H_4O_4Cu$ requires 27·94% Cu.
Sebacate, 23·87% Cu; $C_{10}H_{16}O_4Cu$ requires 24·11% Cu.
Succinate, 35·37% Cu; $C_4H_4O_4Cu$ requires 35·39% Cu.
Sulphanilate, 12·94% Cu, 5·74% N; $C_{12}H_{12}O_6N_2S_2Cu,4H_2O$ requires 13·25% Cu, 5·84% N.
Tartrate, 29·59% Cu; $C_4H_4O_6Cu$ requires 30·04% Cu.

For the preparation of the difficultly soluble salts of amino acids, a clear solution of the acid in water or dilute hydrochloric acid was raised to boiling-point and an excess of cupric oxide or basic carbonate added. The mixture was stirred, boiled for a further 2 min. and filtered through a hot-water funnel. On cooling, the copper salt crystallized out, was filtered off and thoroughly washed with water, alcohol and ether in turn and air-dried. The cystinate was prepared by treating an ammoniacal solution of cystine and copper sulphate with acetic acid:

Aspartate, 23·14% Cu, 5·03% N; $C_4H_5O_4NCu,4\frac{1}{2}H_2O$ requires 23·08% Cu, 5·08% N.
l-cystinate, 20·12% Cu, 8·77% N; $C_6H_{10}O_4N_2S_2Cu$ requires 21·07% Cu, 9·28% N.
Glutamate, 30·19% Cu, 6·64% N; $C_5H_7O_4NCu$ requires 30·47% Cu, 6·71% N.
l-leucinate, 19·74% Cu, 8·51% N; $C_{12}H_{24}O_4N_2Cu$ requires 19·63% Cu, 8·65% N.

β-phenyl alaninate, 14·60 % Cu, 6·59 % N; $C_{18}H_{20}O_4N_2Cu,2H_2O$ requires 14·87 % Cu, 6·55 % N.
l-tyrosinate, 15·26 % Cu, 6·57 % N; $C_{18}H_{20}O_6N_2Cu$ requires 15·01 % Cu, 6·61 % N.
dl-valinate, 21·60 % Cu, 9·43 % N; $C_{10}H_{20}O_4N_2Cu$ requires 21·50 % Cu, 9·47 % N.

Samples of cupric chloride (A.R.), basic carbonate, ferrocyanide, sulphate (A.R.) and cuprous iodide were from laboratory stock but the following derivatives were prepared by special methods:

Basic arsenate. A solution of copper hydroarsenate was obtained by dissolving the basic carbonate in a solution of orthoarsenic acid and, on boiling the clear blue filtrate, the basic arsenate separated out as a light green solid. This was filtered off, suspended in boiling water for ½ hr., filtered, washed with water, alcohol and ether, and dried at 100° C. Found 44·77 % Cu; $Cu_3(AsO_4)_2.Cu(OH)_2$ requires 44·89 % Cu.

Basic carbonate. 5 % cupric sulphate was treated with a slight excess of 5 % sodium bicarbonate solution. The precipitate was washed 5 times by decantation and then at the centrifuge until free from sulphate. The precipitate was resuspended in distilled water and the suspension analysed for copper.

Basic chloride. Attempts to prepare this derivative by the addition of alkali to cupric chloride solutions were frustrated by the high dispersion of the precipitate and use was made of the process given by Marsh & Marsh (U.S.P. 1,937,524). A stream of air (freed from carbon dioxide) was passed through a solution of cupric chloride containing copper foil and heated in a water bath. After 2 days the copper foil had dissolved and the precipitate was filtered off, washed with water and then alcohol. The yield was 102 % calculated on the weight of chloride taken, and the product contained 59·61 % Cu; $CuCl_2,3Cu(OH)_2$ requires 59·50 % Cu.

Basic fluoride. Method A: Cupric fluoride solution, prepared by dissolving pure cupric oxide in dilute hydrofluoric acid and filtering from excess oxide, was boiled. The green precipitate was filtered off, washed with water and then with alcohol, and air-dried. The product gave 61·45 % Cu; $CuF_2,Cu(OH)_2$ requires 63·86 % Cu. Method B: The filtrate from 40 g. commercial sodium fluoride in 250 ml. water was added to 200 g. bluestone and made up to 2 l.; 20 g. copper foil was added, the mixture heated in a water bath and a stream of air (free from carbon dioxide) passed through until the foil had dissolved. The precipitate, a mixture of basic fluoride and basic sulphate, was filtered off, washed with water and then with alcohol and air-dried; 44·2 % Cu.

Basic sulphate. Cupric sulphate solution was treated with 0·75 equivalent of *N* sodium hydroxide. The precipitate, after washing 6 times by decantation and finally on the centrifuge, was resuspended in water and the suspension analysed for copper.

Bordeaux mixture. Prepared from equal weights of bluestone and hydrated lime.

Burgundy mixture. Prepared from bluestone and washing soda, in the weight ratio of 4 : 5.

Cupric oxide (hydrated). Cupric hydroxide, prepared by the precipitation of the sulphate with excess sodium hydroxide, was washed (15–20 times) by decantation until free from sulphate and the colour was greyish black. The copper content of the final suspension was determined.

Cuprous oxide (red). Precipitated by glucose from boiling Fehling's solution. After washing the precipitate 6 times by decantation and twice at the centrifuge, it was resuspended and the copper content of the suspension determined.

Cuprous oxide (yellow). Precipitated by hydroxylamine hydrochloride from cold Fehling's solution; washed and resuspended as for the red form. The preparations of both red and yellow forms were submitted to biological assay without delay.

Cupric sulphide. Precipitated from cupric sulphate solution (acidified with sulphuric acid) by hydrogen sulphide. After filtration and washing with hot water, the sulphide was dried at 100° C., 66·61 % Cu; CuS requires 66·52 % Cu.

Cupric malate. Solutions were prepared by warming solutions of *dl*-malic acid with excess of basic carbonate, but attempts to crystallize the compound from the filtered aqueous solution in the presence of 0·3 mol. malic acid (Pickering, 1913) failed. The solution was therefore treated with alcohol when a sticky green solid was precipitated, which, after filtration and washing with 65 % aqueous alcohol, was dried at 110° C. and ground: 32·79 % Cu; $C_4H_4O_5Cu$ requires 32·49 % Cu.

Cupric cuprimalate. Solutions of cupric malate, prepared as above, after standing 1 week in a stoppered flask, deposited a green solid which, according to Wark (1923), is cupric cuprimalate. The precipitate was filtered off, washed with water and dried to constant weight at 100° C. (37·83 % Cu). On resuspension in water and redrying at 100° C., this product yielded 37·61 % Cu. Separate preparations yielded 37·49, 37·51 % Cu; $Cu(CuC_4H_3O_5)_2,3H_2O$ requires 37·64 % Cu. There is a discrepancy here between the present results and those of Wark (1923) whose product analysed to the pentahydrate, the formula previously adopted by Liebig (1838), who also described a hepta-hydrate. Pickering (1912) isolated a similar green insoluble derivative which he regarded as a basic

salt, $(C_4H_4O_5Cu)_4CuO,6H_2O$, but commented on the poor agreement of his analysis of copper content with that required by this formula. It is possible that Wark and Liebig were describing derivatives of *l*-malic acid, whereas the *dl*-acid was used for the present work.

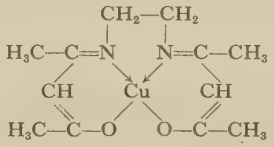
Sodium cuprimalate. Prepared by method described by Wark (1923), 22·19% Cu; $NaCuC_4H_3O_5$, $4H_2O$ requires 21·94% Cu.

Sodium cupritartrate. Prepared by the method described by Packer & Wark (1921), 25·54% Cu; $Na_3Cu_4C_{12}H_9O_{19},11H_2O$ requires 25·99% Cu.

Bis-*ethylenediaminocupric chloride* and *sulphate.* Solutions (0·1% Cu) were prepared when required by the addition of *N*/2 ethylenediamine to cupric chloride and sulphate solutions respectively.

Bis-*ethylenediaminocupric dinitro-o-cresylate.* Precipitated as an orange solid when a solution of cupric sulphate and excess ethylenediamine is treated with a solution of the ethylenediamine salt of dinitro-*o*-cresol, 10·90% Cu, 19·51% N; $(C_7H_5O_5N_2)_2Cu,(CH_2NH_2)_2$ requires 11·01% Cu, 19·40% N.

. *Ethylenediamino*-bis-*acetylacetone, copper salt of.* On cooling a mixture of acetylacetone (2 mol.) and ethylenediamine (1 mol.), a white crystalline mass is formed from which ethylenediamine-*bis*-acetylacetone was recrystallized from water (m.p. 110–111° C.). The copper salt was prepared by precipitating a solution of this derivative with copper acetate, and after recrystallization from alcohol gave m.p. 137° C., in agreement with Morgan & Main Smith (1926) who gave its formula as



For fungicidal tests a weighed amount of the substance was dissolved in 5 ml. acetone in a 250 ml. flask and made up to the mark with water. The suspension so obtained was sprayed immediately after preparation.

Tetra-amminocupric dinitro-o-cresylate. Precipitated by double decomposition of cuprammonium sulphate and the ammonium cresylate, 12·00% Cu, 13·08% NH_3; $(C_7H_5O_5N_2)_2Cu,4NH_3$ requires 12·09% Cu, 12·93% NH_3.

Statistical treatment of the results

In most tests with *Macrosporium sarcinaeforme* reported, the germination of spores on unsprayed slides was sufficiently near 100% to allow a direct conversion of the germination figures to probits. With spores of *Venturia* spp. a correction due to 96–98% germination in the controls was necessary.* The dosage-mortality-regression equation and its error was calculated according to the methods described by Bliss (1935*a*). The estimates of germination in the individual drops per slide were regarded as independent, for they are subject to variation arising from spray (except in the unsprayed slide technique) and drop distribution. The statistics of the regression line together with the calculated LD 50 are assembled in Table 1 in which the symbols used are as follows:

$\bar{x}$ = mean weighted logarithmic dosage;

$\bar{y}$ = mean weighted probit germination;

b = regression of y against x;

n = number of estimates of finite percentage germination less two;

χ^2 = an estimate of the degree to which the observed germination figures depart from those calculated from the regression equation. The values in heavier type are less than those equivalent to $P = 0{\cdot}05$, when the data were regarded as homogeneous;

$V(\bar{y})$, $V(b)$ = the variances of $\bar{y}$ and b respectively, calculated from χ^2.

The copper concentrations are expressed as $\log_{10}\text{Cu} + 3$ and the dimensions of the b value are unit probit/$\log_{10}$ 2. As the value of $\bar{y}$ given in Table 1 is mean weighted germination, the b values are all negative but the minus sign is omitted for convenience. Where the dosage range of the fungicide is inadequate to permit the calculation of the median lethal dose, this dosage is omitted unless there is evidence that the calculated regression line maintains a constant slope beyond the range of the experimental data.

* A full record of the germination figures obtained has been deposited in the Archives established at the Natural History Museum, South Kensington, London, S.W. 7. A second copy is available for consultation at the Agricultural and Horticultural Research Station, Long Ashton, Bristol.

TABLE 1. *Summarized data from all tests*

Compound	LD 50	$\bar{x}$	$\bar{y}$	b	n	χ^2	$V(\bar{y})$	$V(b)$	Curve of case
A. Test organism: *Macrosporium sarcinaeforme*									
(*a*) In vitro method on sprayed slides: log (Cu conc.)+3									
Series I:									
Sebacate	1·248±0·0254	1·2580	4·9011	11·4318	4	9·6813	0·0097	1·7199	
Succinate	1·213±0·1399	1·1352	5·3493	8·0802	4	78·1608	0·0794	3·7686	
Adipate	1·099±0·0270	1·1086	4·9353	7·0887	4	**5·0295**	0·0044	0·1439	
Bordeaux	1·214±0·0970	1·1729	5·1712	5·1071	6	67·8935	0·0322	0·8205	
Series II:									
Bordeaux	0·685±0·0565	0·7196	4·7851	6·9500	6	35·2538	0·0222	0·8406	
Succinate	0·617±0·2237	0·5888	5·0522	6·6375	3	44·3380	0·0607	3·1237	
Tartrate	0·574±0·1159	0·5852	4·9401	5·7842	4	54·8886	0·0542	0·2278	
Oxalate	0·547±0·0572	0·6029	4·7277	5·5372	4	11·9172	0·0101	0·4862	
Adipate	0·521±0·0650	0·6088	4·6603	4·4681	4	**8·8994**	0·0072	0·3400	
Series III:									
Succinate	0·580±0·0794	0·6064	4·842	6·1560	4	33·4455	0·0292	1·384	
Adipate	0·405±0·1126	0·5745	4·291	4·2965	4	11·5905	0·0114	0·648	
Aspartate	0·724±0·0417	0·7568	4·886	3·5757	7	11·2426	0·0038	0·0773	
Bordeaux	0·605±0·0570	0·7553	4·489	3·5700	6	**9·368**	0·0045	0·0987	
Tartrate	0·427±0·0781	0·6737	4·318	2·9384	7	**9·7347**	0·0038	0·0887	
Glutamate	0·578±0·0926	0·7270	4·639	2·7272	7	22·6762	0·0074	0·1440	
Oxalate	0·767±0·0959	0·8303	4·872	2·3162	6	17·7952	0·0067	0·133	
Cystinate	>1·398	—	—	—	—	—	—	—	
Quinaldinate	>1·699	—	—	—	—	—	—	—	
Basic arsenate	>1·699	—	—	—	—	—	—	—	
Series IV:									
Sebacate	0·569±0·0433	0·5504	5·1503	8·9320	4	14·2718	0·0167	1·2282	
Hippurate	0·699±0·0889	0·6785	5·1319	8·6075	2	7·5141	0·0235	1·0902	
Bordeaux	0·808±0·0457	0·8153	4·9610	5·4857	6	16·4543	0·0097	0·3407	
Basic fluoride	0·636±0·1343	0·6455	4·9828	3·2237	4	18·8500	0·0135	0·5968	
Salicylaldoxime	>1·699	—	—	—	—	—	—	—	
Series V:									
Phthalate	0·615±0·0872	0·601	5·086	8·3510	4	40·5906	0·0500	2·43	
Bordeaux	0·899±0·0122	0·889	5·075	7·9043	6	**1·9540**	0·0015	0·0694	
Series VI:									
Basic chloride	0·739±0·0527	0·7677	4·8724	4·6597	7	27·9287	0·0101	0·1951	
Bordeaux	0·824±0·0419	0·8366	4·9482	4·3422	7	16·5266	0·0057	0·1005	
Phosphate	0·451±0·1097	0·6641	4·3251	3·6423	4	12·9912	0·0092	0·2255	
Mucate	1·028±0·0802	1·0788	4·9229	1·5723	13	30·7304	0·0029	0·0176	
Thiocyanate	>1·699	—	—	—	—	—	—	—	
Series VII:									
Glycinate	0·391	0·5393	4·0508	6·4024	1	**0·5101**	0·0027	0·2393	
Alaninate	0·376±0·0858	0·5437	4·1627	6·0165	2	**1·6766**	0·0041	0·3345	
Tyrosinate	0·874±0·0418	0·9346	4·7066	5·0656	5	**8·9574**	0·0057	0·1938	(*a*)
l-leucinate	0·577±0·0646	0·6911	4·6201	3·5827	6	15·1375	0·0066	0·1444	
dl-valinate	0·485±0·0673	0·7384	4·2856	2·9221	7	**10·5441**	0·0035	0·0518	
β-phenylalaninate	0·683±0·0770	0·8859	4·6513	1·7960	10	**16·4961**	0·0026	0·0267	(*b*)
Cystinate	1·474±0·1284	1·0564	5·6898	1·7306	10	19·1355	0·0035	0·0360	(*b*)
Series VIII:									
Bordeaux	0·604±0·0318	0·5833	5·1220	5·8061	7	**10·9269**	0·0059	0·1417	
Basic fluoride (A)	0·916±0·0411	0·8905	5·1194	4·8606	6	11·1999	0·0063	0·1393	
Basic chloride	>1·699	—	—	—	—	—	—	—	
Series IX:									
Glycinate	0·336	0·5916	3·8167	4·4581	1	**0·9906**	0·0044	0·2256	
Sulphate	0·218	0·5778	3·5454	4·0482	1	**1·7791**	0·0106	0·5866	
Alaninate	0·285±0·4121	0·5778	3·5712	4·1662	1	**0·6844**	0·0041	0·2257	
l-leucinate	0·107±0·1504	0·6394	3·9272	2·8546	5	**9·1635**	0·0070	0·1818	
Cystinate	1·417±0·0356	1·3500	5·1876	2·8441	10	**9·4297**	0·0019	0·0255	
dl-valinate	0·444±0·1210	0·7598	4·5760	1·4775	7	**7·0377**	0·0020	0·0351	(*b*)
β-phenylalaninate	—	0·8004	4·3891	1·3710	6	**5·9419**	0·0023	0·0425	(*b*)
Tyrosinate	0·635±0·0784	0·7844	4·8320	1·2145	7	**4·2501**	0·0011	0·0189	(*b*)

TABLE I (*continued*)

Compound	LD 50	$\bar{x}$	$\bar{y}$	b	n	χ^2	$V(\bar{y})$	$V(b)$	Curve of case
Series X:									
Sulphanilate	0·343 ± 0·0228	0·3519	4·9335	7·3273	4	**3·7810**	0·0035	0·1394	
Bordeaux	0·496 ± 0·0602	0·4696	5·1557	6·8637	5	26·4902	0·0215	1·0409	
Lactate	0·479 ± 0·0457	0·5142	4·8066	5·9879	5	14·3108	0·0102	0·3555	
Sulphate	0·516 ± 0·0862	0·4637	5·2450	5·6199	6	58·7301	0·0310	0·8496	
Malonate	0·495 ± 0·0307	0·5025	4·9640	5·0142	7	**10·2970**	0·0041	0·0912	
Alaninate	0·454 ± 0·0781	0·5090	4·8036	3·8263	8	57·5373	0·0150	0·2020	
Glycinate	0·655 ± 0·0571	0·6669	4·9616	3·1979	10	33·6031	0·0064	0·0685	
dl-valinate	0·590 ± 0·0876	0·7080	4·7123	2·5649	6	20·1554	0·0075	0·0444	
Series XI:									
Burgundy	0·523 ± 0·0600	0·4949	5·1148	4·2707	10	56·2681	0·0125	0·1674	
Mucate	0·571 ± 0·0380	0·5706	5·0014	3·9008	12	26·6830	0·0046	0·0539	
Bordeaux	0·613 ± 0·0407	0·5988	5·0487	3·4083	11	22·2033	0·0038	0·0401	
Phosphate	0·690 ± 0·0399	0·6239	5·2018	3·1185	11	**17·4207**	0·0030	0·0323	
Basic chloride	1·787 ± 0·0710	1·5026	5·4783	1·7167	15	**23·4948**	0·0022	0·0130	
Basic arsenate	> 2·0	—	—	—	—	—	—	—	
Thiocyanate	> 2·0	—	—	—	—	—	—	—	
Series XII:									
Burgundy	0·402 ± 0·0335	0·4296	4·8530	5·5771	7	**14·0220**	0·0061	0·1640	(*a*)
Mucate	0·321 ± 0·0333	0·3693	4·8148	3·8911	10	**15·7343**	0·0032	0·0393	
Bordeaux	0·335 ± 0·0291	0·3693	4·8830	3·4495	10	**9·6008**	0·0020	0·0240	
Phosphate	0·508 ± 0·0520	0·5024	5·0195	3·3997	13	45·6608	0·0065	0·0627	
Basic chloride	2·003 ± 0·1077	1·4439	5·7318	1·3368	17	**25·6118**	0·0020	0·0084	
Basic arsenate	> 2·0	—	—	—	—	—	—	—	
Thiocyanate	> 2·0	—	—	—	—	—	—	—	
Series XIII:									
Dinitro-cresylate	0·421 ± 0·0406	0·4458	4·8702	5·3533	5	**8·1401**	0·0047	0·1154	(*a*)
Bordeaux	0·447 ± 0·0347	0·4304	5·0812	4·9949	6	**9.6859**	0·0048	0·1282	(*a*)
Sulphide	0·809 ± 0·0573	0·7881	5·0914	4·4681	6	23·2370	0·0104	0·1981	(*a*)
Cyanide	1·059 ± 0·0469	1·0969	4·9196	2·1703	19	34·4038	0·0023	0·0111	
Series XIV:									
Dinitro-cresylate	0·356	0·3444	5·1552	12·8026	1	26·9244	0·3426	15·1135	
Bordeaux	0·422 ± 0·902	0·4151	5·0317	5·6647	5	46·5184	0·0312	0·9690	
Basic fluoride (B)	Impossible to fit regression line to data								
Series XV:									
Cuprous oxide (yellow)	0·824 ± 0·0430	0·7959	5·1357	4·9555	11	44·7634	0·0090	0·1211	
Bordeaux	0·540 ± 0·0316	0·5373	5·0118	4·5969	10	**16·1745**	0·0042	0·0741	
Cuprous oxide (red)	1·440 ± 0·1250	1·2231	5·6322	3·2231	6	15·1789	0·0070	0·1674	
Basic chloride	1·380 ± 0·0674	1·3080	5·2070	2·9758	14	67·6109	0·0081	0·0646	
Basic carbonate	0·598 ± 0·0727	0·7133	4·7191	2·5358	11	34·3279	0·0061	0·0529	
Basic sulphate	0·295 ± 0·0810	0·6801	4·3900	1·6156	15	**21·4520**	0·0020	0·0116	
Series XVI:									
Bordeaux	0·731 ± 0·0221	0·7265	5·0389	8·0292	5	**6·0072**	0·0047	0·1902	
Sulphate	0·744 ± 0·0222	0·7253	5·1281	6·8226	6	**5·7565**	0·0037	0·1479	
[am]*-dinitro-cresylate	0·701 ± 0·0290	0·6904	5·0685	6·6320	6	**10·1303**	0·0060	0·2110	(*a*)
Chloride	0·985 ± 0·0688	0·9479	5·1075	2·9819	12	61·1739	0·0084	0·0739	
Basic sulphate	1·576 ± 0·0689	1·3866	5·3046	1·6125	17	38·3018	0·0027	0·0012	
[en]*-dinitro-cresylate	> 1·699	—	—	—	—	—	—	—	
Series XVII:									
Chloride	0·535 ± 0·0221	0·5500	4·8740	8·5780	3	**1·5290**	0·0034	0·2495	
[am]-dinitro-cresylate	0·395 ± 0·0122	0·4076	4·1920	8·3610	5	**1·6201**	0·0015	0·0773	
Bordeaux	0·656 ± 0·0496	0·6144	5·2557	6·4433	7	35·2832	0·0167	0·3719	
Basic carbonate	0·789 ± 0·1083	0·8945	4·7222	2·8826	11	105·4929	0·0169	0·1458	
Basic sulphate	—	0·7575	4·2901	0·9612	7	**10·2873**	0·0032	0·0551	
[en]-dinitro-cresylate	> 1·699	—	—	—	—	—	—	—	

* See p. 420.

TABLE I (*continued*)

Compound	LD 50	$\bar{x}$	$\bar{y}$	b	n	χ^2	$V(\bar{y})$	$V(b)$	Curve of case
Series XVIII:									
dl-malate	0·509 ± 0·0217	0·5074	5·0204	7·5216	5	**4·8460**	0·0039	0·1931	
Hippurate	0·620 ± 0·0270	0·6304	4·9287	6·9825	6	**9·3838**	0·0057	0·2024	
Bordeaux	0·597 ± 0·0273	0·5861	5·0681	6·3369	7	**10·2873**	0·0052	0·1806	
Tartrate	0·514 ± 0·0407	0·5130	5·0047	5·9019	6	16·4935	0·0092	0·2730	
Succinate	0·419 ± 0·0220	0·4153	5·0187	5·7705	6	**3·8124**	0·0027	0·0690	
Aspartate	0·760 ± 0·0521	0·7556	5·0166	4·2194	7	23·7845	0·0083	0·1241	
Oxalate	1·599 ± 0·2129	1·0178	5·9362	1·7841	9	25·9486	0·0066	0·0605	
Series XIX*a*:									
Bordeaux	0·649 ± 0·0306	0·6252	5·1200	5·1826	8	**14·2000**	0·0046	0·0858	
Chloride	0·519 ± 0·0459	0·5779	4·7474	4·6396	9	25·8635	0·0080	0·1684	
Basic sulphate	0·726 ± 0·0469	0·8296	4·6874	3·0865	10	18·8607	0·0037	0·0431	
Series XIX*b*:									
Bordeaux	0·479 ± 0·0375	0·4607	5·1316	7·4479	4	**8·8919**	0·0096	0·3120	
Chloride	0·582 ± 0·0538	0·6138	4·8712	4·3018	8	31·1840	0·0095	0·1578	
Basic sulphate	0·730 ± 0·0969	0·9057	4·5295	2·8714	9	49·7233	0·0110	0·1090	
Series XX:									
Phthalate	0·737 ± 0·0568	0·7063	5·1872	6·7066	7	52·2859	0·0234	0·6190	
Bordeaux	0·703 ± 0·0362	0·6783	5·1526	6·4747	7	19·6293	0·0093	0·2733	
Sulphide	1·223 ± 0·0265	1·2164	5·0397	5·7709	7	**9·0666**	0·0041	0·1141	
Lactate	0·741 ± 0·0609	0·7408	5·0019	4·9997	7	41·1672	0·0157	0·2225	
Glutamate	0·808 ± 0·0746	0·8024	5·0256	4·8911	4	27·0291	0·0158	0·2644	
Basic fluoride	0·802 ± 0·0616	0·7959	5·0265	4·4463	8	48·1256	0·0134	0·1791	
Cyanide	> 1·699	—	—	—	—	—	—	—	
Series XXI:									
Sulphate	0·680 ± 0·0116	0·6905	4·9308	6·6443	8	**12·5640**	0·0058	0·2064	
Chloride	0·777 ± 0·0319	0·7796	4·9861	6·1021	7	14·1694	0·0068	0·0207	
[en]-sulphate	1·534 ± 0·0214	1·5235	5·0365	3·4791	13	**7·4403**	0·0012	0·0143	
[en]-chloride	1·545 ± 0·0255	1·5235	5·0735	3·3783	13	**9·8635**	0·0016	0·0190	
Series XXII:									
Malate	0·375 ± 0·0167	0·3805	4·9547	8·8143	4	**2·3559**	0·0028	0·1298	
Cuprimalate	0·366 ± 0·0326	0·3420	5·1678	7·2922	5	**8·4479**	0·0081	0·3035	
Bordeaux	0·328 ± 0·0335	0·3357	4·9523	6·7496	6	13·5550	0·0082	0·3195	
Sodium cuprimalate	0·363 ± 0·0231	0·3726	4·9398	6·5138	7	**8·1949**	0·0040	0·1358	
Sodium cupri-tartrate	0·494 ± 0·0526	0·4992	4·9738	5·9373	7	38·4965	0·0163	0·3983	
Series XXIII:									
Bordeaux	0·697 ± 0·0249	0·7205	4·8941	4·5970	7	**5·9149**	0·0023	0·0346	
Basic sulphate	0·668 ± 0·0275	0·8669	4·4727	2·6662	19	**15·1422**	0·0008	0·0103	
Basic chloride	1·819 ± 0·0662	1·5197	5·5486	1·8031	14	**19·3700**	0·0020	0·0114	
Iodide	> 2·000	—	—	—	—	—	—	—	
Series XXIV:									
Phthalate	0·450 ± 0·0364	0·4216	5·2042	7·5523	6	19·0052	0·0117	0·4384	
dl-malate	0·801 ± 0·0264	0·7947	5·0355	6·1518	7	**9·3053**	0·0044	0·1329	
Tartrate	0·749 ± 0·0531	0·7370	5·0676	5·8924	7	38·6566	0·0164	0·3885	
Benzoate	0·927 ± 0·0379	0·8964	5·1534	5·1713	10	26·6334	0·0074	0·1513	
Bordeaux	0·619 ± 0·0357	0·6164	5·0118	4·7017	9	**17·3837**	0·0054	0·1122	
Glutamate	0·777 ± 0·0535	0·7860	4·9628	4·3388	10	36·7125	0·0103	0·1768	
Series XXV:									
Bordeaux	0·706 ± 0·0308	0·6862	5·1147	5·9794	8	**14·4403**	0·0061	0·1949	
[ec]*	0·648 ± 0·0354	0·7486	4·6404	3·6139	6	**5·3505**	0·0023	0·0336	
Ferrocyanide	> 2·000	—	—	—	—	—	—	—	
Basic chloride	> 2·000	1·6675	5·7666	1·8872	11	**8·5252**	0·0017	0·0141	
Series XXVI:									
Bordeaux	0·506 ± 0·0387	0·5407	4·8584	4·1914	10	22·1089	0·0051	0·804	
Basic carbonate	0·962 ± 0·0269	0·9778	4·9434	3·7146	12	**11·1318**	0·0021	0·0220	
Cupric oxide	0·917 ± 0·0297	0·9709	4·8209	3·3351	9	**8·1140**	0·0018	0·0219	

* See p. 420.

TABLE I (*continued*)

Compound	LD 50	$\bar{x}$	$\bar{y}$	b	n	χ^2	$V(\bar{y})$	$V(b)$	Curve of case
Series XXVII:									
[ec]	0·606 ± 0·0444	0·661	4·7370	5·0513	7	18·6675	0·0079	0·2277	
Bordeaux	0·751 ± 0·0238	0·7317	5·0896	4·6897	9	**7·6740**	0·0022	0·0393	
Cuprous oxide (yellow)	1·010 ± 0·0332	0·9811	5·0897	3·1704	13	**16·2746**	0·0023	0·0226	
Cupric oxide	1·331 ± 0·0484	1·1238	5·5342	2·6328	9	**8·4513**	0·0020	0·0269	(*b*)
Basic carbonate	1·097 ± 0·0801	1·0969	5·0004	1·7625	18	78·2820	0·0045	0·0168	
Cuprous oxide (red)	> 2·000	—	—	—	—	—	—	—	
Series XXVIII:									
Bordeaux	0·431 ± 0·0744	0·4654	4·7949	7·0928	6	60·3887	0·0373	1·4055	
Cuprous oxide (yellow)	0·663 ± 0·0393	0·6684	4·9783	3·9703	12	22·8145	0·0049	0·0884	
Cuprous oxide (red)	> 2·000	—	—	—	—	—	—	—	
Series XXIX:									
Bordeaux	0·395 ± 0·0474	0·4138	4·9000	5·5403	7	26·4317	0·0115	0·3188	
Basic carbonate	0·472 ± 0·0391	0·5751	4·7312	2·6601	11	**12·3922**	0·0020	0·0193	
Cuprous oxide (red)	2·484 ± 0·0503	2·2022	5·6145	2·2447	12	28·4556	0·0004	0·0288	
Series XXX:									
Tartrate	0·370 ± 0·0467	0·4078	4·8414	4·5763	7	19·9475	0·0076	0·1709	
Bordeaux	0·635 ± 0·0456	0·6383	4·9879	4·2271	10	30·0153	0·0072	0·1162	
Series XXXI:									
Sodium cuprí-tartrate	0·088 ± 0·0873	0·2253	4·3453	6·3532	3	**5·0540**	0·0085	1·0025	
Cuprimalate	1·837 ± 0·4464	0·2564	4·3562	5·9636	3	29·0177	0·0414	2·6093	
Sodium cuprimalate	0·077	0·2462	4·1306	5·1292	3	25·5866	0·0413	3·1634	
Bordeaux	0·619 ± 0·0556	0·6224	4·9847	4·1607	10	44·1800	0·0103	0·1561	
Malate	< 0·194	—	—	—	—	—	—	—	
Series XXXII:									
Malate	0·203 ± 0·0333	0·1938	5·0533	5·7474	8	19·3932	0·0067	0·1355	
Sodium cuprimalate	0·538 ± 0·0281	0·5354	5·0141	5·3768	8	**12·2141**	0·0042	0·0858	
Cuprimalate	0·536 ± 0·0421	0·5191	5·0747	4·4234	11	34·4888	0·0070	0·0934	
Series XXXIII:									
Sodium cuprí-tartrate	0·290 ± 0·0293	0·2843	5·0236	4·5420	10	**14·6924**	0·0035	0·0551	
Tartrate	0·329 ± 0·0886	0·5342	4·1953	4·2355	6	22·1198	0·0129	0·2253	
Series XXXIV:									
Malate	0·337 ± 0·0291	0·3373	5·0005	5·0333	14	39·6074	0·0046	0·1082	
Sodium cuprimalate	0·162 ± 0·0397	0·1875	4·8806	4·9374	15	90·1899	0·0084	0·1846	
Sulphate	0·596 ± 0·0253	0·5820	5·0658	4·6811	20	49·0866	0·0032	0·0582	
Cuprimalate	0·024 ± 0·0328	0·1152	4·6338	4·0988	15	29·9675	0·0031	0·0931	

(*b*) In vitro method on unsprayed slides: log p.p.m. Cu

Series (*a*):									
Sulphate	0·573	0·6221	4·5958	8·1926	1	4·6145	0·0213	0·7130	
Sulphanilate	0·594 ± 0·0366	0·6120	4·8647	7·6380	3	**4·2150**	0·0073	0·2710	
Malonate	0·553 ± 0·0436	0·5449	5·0517	7·1634	4	12·3816	0·0117	0·4635	
dl-valinate	1·368 ± 0·0309	1·2982	5·1973	2·8324	10	**7·8260**	0·0015	0·0128	
Glycinate	1·091 ± 0·0680	1·1877	4·8166	1·9797	10	19·2363	0·0032	0·0339	(*c*)
Alaninate	0·875 ± 0·0249	1·3001	4·2591	1·7895	5	**1·2518**	0·0008	0·0121	(*c*)
Series (*b*):									
Sulphate	0·430 ± 0·0197	0·4466	4·8614	8·5412	9	20·8724	0·0053	0·3357	
Glycinate	1·007 ± 0·0299	1·0105	4·9932	2·4152	31	49·8993	0·0012	0·0755	
Malonate	> 0·625	1·0178	4·0779	2·3467	16	46·4769	0·0039	0·0588	(*c*)
Alaninate	0·985 ± 0·0286	1·1890	4·6935	1·5096	22	**8·7491**	0·0003	0·0030	(*c*)

TABLE 1 (*continued*)

Compound	LD 50	$\bar{x}$	$\bar{y}$	b	n	χ^2	$V(\bar{y})$	$V(b)$	Curve of case
B. Test organism: *Venturia inequalis*. In vitro method on sprayed slides: log (Cu conc.)+3									
Series I:									
Adipate	$\bar{1}\cdot894\pm0\cdot0813$	$\bar{1}\cdot9073$	4·9507	5·0405	7	70·4241	0·0265	0·5167	
Tartrate	$0\cdot590\pm0\cdot0295$	0·0503	5·0421	4·9384	6	**7·3064**	0·0035	0·0799	(*a*)
Sebacate	$0\cdot005\pm0\cdot0899$	0·0170	4·9551	4·6155	7	71·6139	0·0266	0·5038	
Phthalate	$\bar{1}\cdot864\pm0\cdot0574$	$\bar{1}\cdot8511$	5·0552	4·4694	6	25·2028	0·0103	0·2176	
Succinate	$\bar{1}\cdot976\pm0\cdot0414$	0·0015	4·8912	4·3464	6	13·9400	0·0052	0·0828	
Aspartate	$\bar{1}\cdot980\pm0\cdot0303$	$\bar{1}\cdot9741$	5·0185	3·4213	9	**7·7759**	0·0021	0·0266	
Mucate	$0\cdot117\pm0\cdot0269$	$\bar{1}\cdot9081$	5·6262	3·0206	7	**1·5750**	0·0006	0·0132	(*b*)
Bordeaux	$0\cdot343\pm0\cdot0532$	0·2536	5·2826	3·2211	10	32·2814	0·0054	0·0475	
Oxalate	$0\cdot329\pm0\cdot0955$	0·1079	5·5754	2·7936	9	36·0165	0·0081	0·1059	(*b*)
Glutamate	$0\cdot039\pm0\cdot0979$	$\bar{1}\cdot8449$	5·4132	2·4521	7	19·4121	0·0059	0·1079	(*b*)
Basic fluoride (A)	Probits fall in two lines of different slope (see Text-fig. 1)								
Series II:									
Sulphate	0·168	0·2209	4·5511	8·4525	1	**1·5131**	0·0073	0·9859	
Sulphanilate	0·192	0·2557	4·4955	7·7405	1	**1·5417**	0·0096	0·6488	
Malonate	$0\cdot266\pm0\cdot0640$	0·3896	4·5631	3·8158	5	11·3602	0·0062	0·1548	
Alaninate	$0\cdot185\pm0\cdot0827$	0·4374	4·3789	2·5912	9	17·4469	0·0045	0·0666	
Glycinate	$0\cdot198\pm0\cdot0719$	0·4697	4·3850	2·3421	9	**11·7601**	0·0028	0·0363	
dl-valinate	$>0\cdot213$	0·7853	3·9807	1·9192	8	**14·7899**	0·0043	0·0496	(*c*)
C. Test organism: *Venturia pirina*. In vitro method on sprayed slides: log (Cu conc.)+3									
Tartrate	$\bar{1}\cdot833\pm0\cdot0567$	$\bar{1}\cdot9446$	4·5298	4·5172	4	**7·7758**	0·0059	0·1786	
Bordeaux	$\bar{1}\cdot860\pm0\cdot0639$	$\bar{1}\cdot9605$	4·6120	4·2239	6	18·8086	0·0089	0·2434	
Basic fluoride (A)	$\bar{1}\cdot955\pm0\cdot0750$	$\bar{1}\cdot9877$	4·8194	4·0550	5	21·0019	0·0115	0·3173	
Glutamate	$\bar{1}\cdot952\pm0\cdot0790$	0·0232	4·6468	3·6748	6	26·3451	0·0104	0·2198	
Mucate	$\bar{1}\cdot803\pm0\cdot1443$	$\bar{1}\cdot9592$	4·5731	3·6581	3	14·3470	0·0145	0·3328	
Succinate	$\bar{1}\cdot860\pm0\cdot0740$	$\bar{1}\cdot9943$	4·5620	3·5414	6	18·1877	0·0077	0·1704	
Phthalate	$0\cdot013\pm0\cdot0527$	0·0852	4·6671	3·4013	6	**11·5226**	0·0046	0·0575	
Adipate	$\bar{1}\cdot530\pm0\cdot4372$	0·0872	4·0482	2·9937	3	23·4300	0·0292	0·3801	
Aspartate	$0\cdot096\pm0\cdot0526$	0·1922	4·6900	2·7089	10	23·3702	0·0039	0·0357	
Oxalate	$0\cdot053\pm0\cdot0800$	0·3167	4·6464	1·3803	13	**18·3809**	0·0018	0·0111	

The [χ^2] values in heavy type indicate homogeneity.
* [am] = ammino-.
[ec] = dibasic acid radical of ethylenediamino-*bis*-acetylacetone.
[en] = ethylenediamino-.

The dosage-germination curves

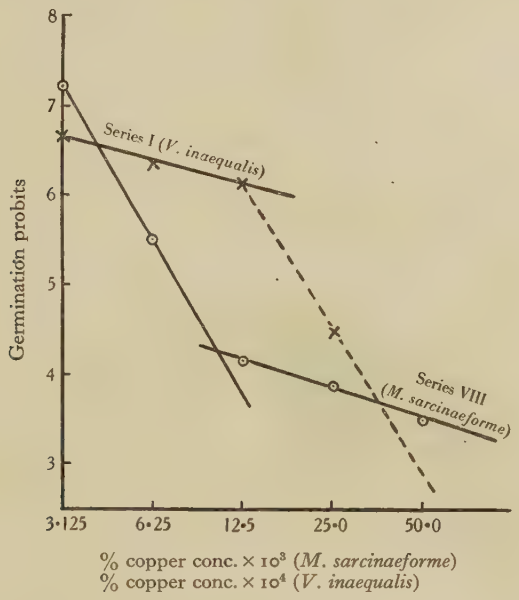
Of the fifty compounds tested, seven did not affect spore germination to an extent that permitted the calculation of a mortality dosage curve over the range of concentrations used. These compounds, which include the basic arsenate, ferrocyanide, quinaldinate, the copper salt of salicylaldoxime, cuprous iodide and thiocyanate, may therefore be regarded as non-fungicidal. These six compounds are characterized by a high degree of stability and insolubility, a sufficient explanation of their lack of toxicity. The promising results obtained by Waters *et al.* (1939) with the basic arsenate are perhaps due to their tests being on fungi in agar culture.

Departures from single linearity

Most of the remaining compounds yielded results which permitted the calculation of a single linear relationship between probit germination and the logarithm of the copper concentration. An outstanding exception was the basic fluoride which, in series VIII and XIV with *Macrosporium sarcinaeforme* and in series I with *Venturia inaequalis*, did not

yield a simple linear relationship. The data, in series VIII and I, in which the purer compound (method A) was used, could best be interpreted by two straight lines of different slope as indicated in Fig. 1. In the case of series XIV, in which the mixture of basic fluoride and basic sulphate prepared by method B was used, insufficient data were obtained to permit an approximation to the shape of the dosage-germination curve.

Less obvious indications that the points were fitted better to the straight lines of different slopes were obtained with other compounds in a limited number of cases, when the probit germinations at the concentration giving approximately 0 or 100% germination fell above or below the linear relationship common to results at other concentrations. The experimental evidence of a departure from single linearity can be expressed by degree of reduction of



Text-fig. 1. Dosage-germination curve of basic fluoride.

the χ^2 value obtained when the germination figures at one or other extreme of the concentration range giving between 0 and 100% germination are ignored. All the examples met are tabulated in Table 2.

Three types of abnormality may be distinguished: case (*a*), when the germination at the lowest concentration is less than that expected; case (*b*), when germination at the higher concentration is less than expected; case (*c*), when germination at low concentrations is greater than expected.

Case (*a*). The germination figures at the lowest concentration are less than would be expected if a single line represented the probit germination-dosage curve, as in that given in Fig. 1 for series I (*V. inaequalis*) but with points of one concentration only on the line of less steep slope. Seven examples were observed, in six of which the omission of the aberrant concentration has rendered the results homogeneous. The explanation of this phenomenon, which is illustrated by the results graphically expressed by McCallan &

Wilcoxon (1936) with *Glomerella cingulata*, is probably to be found in the Arndt-Schutz Law that low concentrations of a toxicant act as a stimulant. It would appear that there is a critical concentration of copper which must be exceeded before the mortality-dosage curve becomes normally distributed.

Case (*b*). The germination figures at the highest concentration are less than would be expected if the probit germination-dosage curve were one straight line. In six of the nine examples the omission of the estimates at the highest concentration has reduced χ^2 to values below those corresponding to $P=0{\cdot}05$. The simplest explanation of this phenomenon is that, at the higher concentration, the effects of a second toxic constituent become observable, being masked at lower concentrations by the greater effect of the toxic constituent responsible

TABLE 2. *Departure from simple linearity in the probit germination-dosage curve*

		All results			Ignoring aberrant results		
		b	χ^2	*n*	*b*	χ^2	*n*
Case (*a*):							
Series VII:	Tyrosinate	3·5299	48·0957	8	5·0656	**8·9574**	5
Series XII:	Burgundy	4·7614	41·6763	10	5·5771	**14·0220**	7
Series XIII:	Bordeaux	4·4222	27·1862	9	4·9949	**9·6859**	6
	Dinitrocresylate	4·4967	33·4295	8	5·3533	**8·1401**	5
	Sulphide	3·9513	62·6450	9	4·4681	23·2370	6
Series XVI:	[am]-dinitrocresylate	5·6052	40·6534	9	6·6320	**10·1303**	6
Series I: (*V. inaequalis*)							
	Tartrate	4·4255	23·6054	9	4·9384	**7·3064**	6
Case (*b*):							
Series VII:	Cystinate	2·1835	36·2977	13	1·7306	19·1355	10
	Phenylalaninate	2·0547	26·6528	10	1·7960	**16·4961**	10
Series IX:	Tyrosinate	1·9813	36·5166	10	1·2145	**4·2501**	7
	Valinate	1·9926	22·4002	8	1·4775	**7·0377**	7
	Phenylalaninate	1·9153	39·8921	6	1·3710	**5·9419**	6
Series XXVII:	Cupric oxide	3·2321	33·3997	12	2·6328	**8·4513**	9
Series I: (*V. inaequalis*)							
	Oxalate	3·3230	49·9913	10	2·7936	36·0165	9
	Glutamate	3·2453	44·6240	9	2·4521	19·4131	7
	Mucate	3·8833	22·9626	7	3·0206	**1·5750**	7
Case (*c*):							
Series (*a*):	Glycinate	2·4009	52·0802	12	1·9797	19·2363	10
	Alaninate	3·5293	68·5275	9	1·7895	**1.2518**	5
Series (*b*):	Alaninate	2·0608	86·0124	28	1·5096	**8·7491**	22
	Malonate	3·7052	125·0133	21	2·3467	46·4768	16
Series II: (*V. inaequalis*)							
	Valinate	2·4336	32·9071	9	1·9192	**14·7899**	8

for the line of less steep slope. Experimental verification of this hypothesis was obtained by examining the toxicity of a sample of the ferrocyanide from which soluble copper had not been completely washed. The curve obtained was of the type illustrated in Fig. 1 with *Venturia inaequalis* and by Bliss (1939) for hypothetical mixtures of the toxic ingredients of independent joint action. On this hypothesis there is no reason to suppose that the appearance of the line of steeper slope will be deferred until the highest concentration yielding germination figures. That this did occur in the examples cited in Table 2 is due to the relatively large dosage ratio of two, and it is probable that the example of the tyrosinate placed in series (*a*) is parallel to that of the tyrosinate results placed in series (*b*) except that, in the former example, the effects of the toxic ingredient responsible for the line of steeper slope have become apparent at a lower concentration than in the latter example. The implications of this suggestion are dealt with in the discussion of the mode of action of the complex derivatives.

The curves of cases (*a*) and (*b*) are of the type described by McCallan *et al.* (1941) as of double slope curvatures concave upwards, but examples were also met of convex upwards type as in case (*c*).

Case (*c*). The germination at the lowest concentration was greater than would be expected if the curve were of constant slope. Of the five examples met, the omission of the aberrant values rendered the results homogeneous in three examples, and in four cases the lowest concentration only was involved. Nevertheless, the results on *Macrosporium sarcinaeforme* suggest that, as in case (*b*) above, the point of intersection of the two lines may occur at any concentration within the range giving finite probit germinations. If so, no satisfactory explanation can be advanced to account for the apparent disappearance from the toxic reaction of the compound responsible for the line of steeper slope which dominates at the lower concentrations. McCallan *et al.* (1941), who noted pronounced examples of this phenomenon with zinc chloride, suggested that an antagonistic action between the zinc and hydrogen ions resulted in a changed distribution of resistances above a certain concentration. In the examples of Table 2 and Fig. 1 it is possible to postulate the presence of more than one toxic constituent permitting the phenomenon to be attributed to a chemical cause rather than to the presence, in the spore population, of two or more levels of resistance, which would, presumably, be apparent in all tests with samples of that particular population.

Normal linear relationships

Cases (*a*), (*b*) and (*c*) are the exception rather than the rule and, in the remainder of the experiments, a single linear relationship between probit germination and logarithm of the copper concentration is apparent. In 43 % of the tests the value of χ^2 is lower than that corresponding to $P=0{\cdot}05$ and it may be accepted that, in these tests, the divergence of the observed germination figures from those calculated from the regression equation is due solely to expected fluctuations in sampling. In most cases in which the recorded χ^2 value is higher than that corresponding to $P=0{\cdot}05$ it is due to fluctuation between the germination figures at individual dosages and is, therefore, associated with either a non-uniform distribution of the spray deposit or irregularities in conditions between the spore droplets. In only 4 % of the tests was the χ^2 figure too large or the number of observations ($=n+2$) too small to justify the calculation of the limits of error ($P=0{\cdot}05$) of the median lethal dose. This calculation is of no value in cases (*a*), (*b*) and (*c*) when the five-probit ordinate cuts the line ignored in the calculation of the regression equation. In these circumstances the dosage on the calculated line is given with an indication that the true dosage is greater or less than this figure. In no case was the distribution of the plotted points such as would indicate that a better linear relationship would be obtained by substituting some function other than the logarithm of the copper concentration as abscissa; the index of variation (Parker-Rhodes, 1942) is zero.

This general conclusion may be compared to the results of other investigations. McCallan *et al.* (1941) recorded that with *Macrosporium sarcinaeforme* the basic chloride, basic sulphate, Bordeaux mixture, cuprous oxide, copper phosphate and copper zeolite gave linear mortality-log. concentration curves; copper hydroxide and copper ammonium silicate gave convex curves, i.e. of case (*c*). On the other hand, copper sulphate and, in a more detailed test, Bordeaux mixture gave concave curves. From their Fig. 4 it would seem that these two compounds yield results of case (*b*) type, but it is difficult to postulate the presence of two independent toxic constituents in these sprays. Rather would it appear both from this Fig. 4 and from the graphical representation of their results with Bordeaux mixture on spores of *Glomerella cingulata* (Fig. 1 B) that there is a linear relationship between probit

mortality and copper concentration, i.e. the index of variability is 1. A second example of this change in the index of variability is referred to on p. 433. Heuberger & Horsfall (1939) also presented data on the toxicity of various samples of cuprous oxides to spores of *M. sarcinaeforme* which permit of statistical examination. In all cases a linear relationship between probit mortality and the logarithm of the dosage is obtained as indicated by the low values of χ^2 in Table 3. A similar conclusion was reached by Parker-Rhodes (1941) in his tests of various copper compounds on spores of this fungus or of *Botrytis allii*.

TABLE 3. *Constants of regression lines calculated from data of Heuberger & Horsfall* (1939)

Sample and experiment no.	$\bar{x}$	$\bar{y}$	LD 50 mg./cm.^{2}	b	n	χ^2	$V(b)$	Mean particle diam. (μ)
				Series *a* (Table 3)				
T 1	2·0070	4·9906	102	2·7126	4	15·5225	0·1891	2·93
R 4	1·6400	5·5369	27	2·6134	4	**7·3833**	0 1027	1·94
R 8	1·5780	5·2564	33	4·2356	1	**0·1432**	0·0279	1·47
Y 1	1·1990	5·6180	11	3·6859	2	23·3772	1·8302	0·94
				Series *b* (Table 4)				
R 6	1·4227	4·6885	42	1·5892	1	**0·3792**	0·0296	2·57
R 7	1·4481	4·8866	31	2·6098	1	**0·2298**	0·0228	1·87
R 8	1·3625	5·2652	18	2·6868	1	**0·0805**	0·0068	1·47
				Series *c* (Table 5)				
T 1	2·0418	5·0815	102	2·4722	4	24·7121	0·2809	2·93
T 1 (*a*)	2·0665	4·8791	132	2·2382	4	15·6605	0·2138	—
T 1 (*b*)	1·9095	5·5728	54	3·0401	4	9·5179	0·1407	—
T 1 (*c*)	0·9131	4·6354	13	1·8616	4	**4·9589**	0·0449	—
Mean	—	—	—	2·6669	10	144·3902	0·0055	—

The characterization of fungicidal value

The reduction of the results of the biological assay to a linear relationship permits the characterization of fungicidal value by two statistics, one defining the position of the regression line, the other its slope. The former value is conveniently taken as the median lethal dose (LD 50), the latter is given by the regression coefficient, *b*.

The regression coefficient

This coefficient, being the reciprocal of the standard deviation of the logarithms of the individual effective doses, gives a measure of the uniformity with which the individual spores react to the fungicide. If, therefore, in a series of tests with a given spore population, different compounds yield similar *b* values, it may be inferred that these compounds are similar in their mode of fungicidal action. It follows, also, that a series of tests with a given spore population and with samples of a given compound which differ, for example, in degree of fineness of particle, should yield similar *b* values provided that these are on a logarithmic basis and that the coarseness of the particles is not so great that the logarithm of the individual effective doses ceases to be normally distributed. This inference is in accordance with the results obtained by Heuberger & Horsfall (1939) assembled in Table 3. Whereas the median lethal doses of the various samples of cuprous oxide vary from 11 to 132 mg. Cu/cm.$^2 \times 10^4$ and follow the mean particle diameter, the regression coefficients are of the same order and are independent of particle size. The high χ^2 value (144·4) of the mean regression coefficient reveals heterogeneity due largely to the wide difference between the *b* values for sample R8 in series *a* and *b*.

The relative independence of the regression coefficient on particle size and, according to Parker-Rhodes (1941), on variations in environmental factors, renders it of particular value in the comparisons of the fungicidal action of relatively insoluble derivatives such as used in these tests, for its use eliminates differences associated with particle size. But of greater value is its use as a quantitative measure of the property which previously (Horsfall *et al.* 1937) was called the 'inherent toxicity'. Parker-Rhodes (1941, 1942) has developed this hypothesis, which he terms the theory of variability, on more general lines. From the relative magnitudes of the variability of a given population of spores to different compounds he is able not only to select the probable permeative radical able to penetrate the spore wall, but also to suggest the reaction by which this 'inherent toxicant' is formed from the fungicide under test. The general definition of variability, $W_\alpha(x)$, may be expressed mathematically as follows:

$$W_\alpha(x) = V(x^\alpha)/\alpha^2\bar{x}^{\alpha 2},$$

which has, at the limit $\alpha = 0$, the value

$$W_0(x) = V(\log_e x),$$

and is estimated by the reciprocal of the square of the regression coefficient of probit mortality against the Napierian logarithm of concentration.

The question arises whether, in tests involving different spore populations, a consistent value of the regression coefficient can be obtained for a given compound. McCallan *et al.* (1941) concluded that the slope of a given toxicity curve is reasonably consistent in replicate tests, whilst Parker-Rhodes (1941) attributed heterogeneity arising in the combination of tests each homogeneous to fluctuations in the LD 50 value, and eliminated heterogeneity by an adjustment of each dosage by a constant factor. A direct comparison of the b values independently of the median lethal doses is, however, possible but the method given by Bliss (1935*b*) is not applicable because all the values quoted in Table 1 are not from homogeneous data. We are indebted to Dr F. Yates (*in litt.* 1941) for an extension of this method to heterogeneous data. If each 'b' value is weighted by the reciprocal of its variance, then

$$\bar{b} = \Sigma Wb/\Sigma W; \quad V(\bar{b}) = 1/\Sigma W.$$

A test of the agreement between the different estimates of the regression coefficient may be made by $\chi^2 = \Sigma Wb^2 - (\Sigma Wb)^2/\Sigma W$ with degrees of freedom one less than the number of estimates.

When this process is applied to the twenty-nine estimates of the regression coefficient for Bordeaux mixture against *Macrosporium sarcinaeforme* recorded in Table 1, a χ^2 value of 445·0725 results, indicating a wide departure from homogeneity. Inspection of the data shows that the values obtained in series III, XI and XII, are abnormally low; those from V and XVI are abnormally high. If these estimates are ignored χ^2 is reduced to 105·3953 which, with 23 degrees of freedom, is not excessively greater than that required for homogeneity. It may therefore be concluded that the technique employed for obtaining the spore suspension of *M. sarcinaeforme* yields populations sufficiently standard to give generally consistent values of the regression coefficient of a given compound. If, on the one hand, a wide divergence is obtained, an explanation is to be sought in the chemistry of the compound under test rather than in spore variations. If, on the other hand, a close comparison of the regression coefficients of different compounds is required, it is better to compare estimates obtained with a given spore population rather than to rely on the mean values obtained with different samples of spores.

The mean values of the regression coefficients of the different compounds tested are assembled in Table 4. In eighteen of the forty-two means, the χ^2 value reveals no evidence of heterogeneity, though, in five cases, one estimate of the regression coefficient has been ignored in the calculation of the mean. The cases of high χ^2 values are more conveniently discussed under the specific compound tested, but two general points emerge. The first is that when the spore suspension is added direct to the diluted fungicide (series (*a*), (*b*)) instead of being placed on the dried spray deposit (series I–XXXIV), a different regression coefficient may result; that for the sulphate is significantly greater, that for the alaninate is significantly smaller on the unsprayed slide. One explanation is that, with soluble derivatives, diffusion adds to the factors responsible for variability in spore germination on the sprayed slide but not on the unsprayed slide. A higher regression coefficient is therefore to be expected on the unsprayed slide, unless other factors associated, for example, with the drying of the spray deposit intervene. The second general point is that, on the sprayed slides, ten of the fifteen estimates of *b* omitted in Table 4 occur in series I–VII. In these early series the spore suspension was not thoroughly washed to remove nutrient material as in the later series, a difference in technique which may complete the explanations which follow of the irregularities noticed.

The median lethal dose

The estimates of median lethal dose of a given compound in different series show some degree of consistency. For example, the LD 50 of Bordeaux mixture varies between 2·13 (series XXII) and 7·92 (series V) mg./100 ml., series I yielding the exceptionally high figure of 16·37 mg./100 ml. The otherwise consistent results may be due to a broad correlation between the median lethal dose and the regression coefficient which is shown in Table 1 by the tendency of the LD 50's in any series to increase as the series is descended, the compounds in each series being arranged in decreasing values of the regression coefficient. McCallan *et al.* (1941) also found a highly significant correlation between steepness of slope and toxicity at the LD 50 point for heavy metal and copper compounds exhibiting a linear probit germination-log concentration relationship. Yet, it would be expected that a compound showing a low regression coefficient and, therefore, of low inherent toxicity would require a relatively high median lethal dose. The correlation arising from this cause would be masked in comparisons of compounds of different availability, as in the tests of different samples of cuprous oxide recorded in Table 3. With compounds of low solubility, comparisons of LD 50 are therefore unlikely to yield trustworthy results unless particle size data are taken into consideration but the figure, if exceptional, may provide clues to mode of action.

The fungicidal value of individual compounds

A consideration of the fungicidal value of individual compounds as indicated by their respective regression coefficients and median lethal doses is simplified by their classification into groups.

Un-coordinated compounds of copper

Simple cupric salts. Of the soluble salts, **cupric chloride** ($\bar{b} = 5{\cdot}9445$) would appear to have a greater inherent toxicity than **cupric sulphate** ($\bar{b} = 5{\cdot}2728$), but in direct comparison (series XXI) and in comparisons through Bordeaux mixture (see below) no difference is shown. Both yield mean regression coefficients lower than that of the **sulphanilate**

($\bar{b}=7{\cdot}4328$) which, in series X, yields a lower LD 50 than the sulphate. But when added direct to the spore suspension (series *a*) or against *Venturia inaequalis*, no difference appears, and it is doubtful whether the apparent superiority of the sulphanilate is real. For this reason it has been included in the unco-ordinated group.

TABLE 4. *Mean values of regression coefficients*

Compound	Estimates omitted	$\bar{b}$	$V(\bar{b})$	χ^2	n
Sebacate	—	9·9733	0·7163	**2·1204**	1
[am]*-dinitrocresylate	—	7·7322	0·0597	21·6963	1
Sulphanilate	—	7·4328	0·0921	**0·2355**	1
Phthalate	—	7·3114	0·2321	**1·1697**	2
Hippurate	—	7·2370	0·1707	**2·0423**	1
Chloride	XVI	5·9445	0·0155	58·9302	3
Malate	XXII	5·9426	0·0341	20·5263	3
Succinate	—	5·8063	0·0644	**0·3375**	1
Sodium cuprimalate	—	5·6157	0·0404	9·1763	3
Dinitrocresylate	—	5·4327	0·1428	**3·6401**	1
Lactate	—	5·3801	0·1368	**1·6896**	1
Malonate	(*b*)	5·3676	0·0762	8·3241	1
Sulphide	—	5·2948	0·0724	5·4336	1
Sulphate	(*a*), (*b*)	5·2728	0·0306	61·8960	4
,,	(*a*) and (*b*) only	8·4296	0·2282	**0·1162**	1
Benzoate	—	5·1713	—	—	—
Cuprimalate	—	5·0712	0·0505	21·1458	3
Bordeaux	III, V, XI, XII, XVI	5·0053	0·0063	105·3953	23
Tartrate	III	4·9563	0·0497	**8·5970**	4
Burgundy	—	4·9306	0·0828	5·1502	1
Sodium cupritartrate	—	4·7871	0·0462	6·8616	2
Glutamate	III	4·5601	0·1060	**0·6922**	1
Basic fluoride	—	4·5104	0·0693	3·6780	2
Alaninate	(*a*), (*b*)	4·4773	0·0808	9·6105	2
,,	(*a*) and (*b*) only	1·5652	0·0024	5·1944	1
Adipate	I	4·4090	0·2230	**0·0290**	1
Mucate	VI	3·8952	0·0227	**0·0000**	1
Aspartate	—	3·8228	0·0476	**2·0575**	1
[ec]*	—	3·7987	0·0293	7·9110	1
Cuprous oxide (yellow)	—	3·6092	0·0157	11·2752	2
[en]*-sulphate	—	3·4791	0·0143	—	—
[en]-chloride	—	3·3783	0·0190	—	—
l-leucinate	—	3·2604	0·0805	**1·6248**	1
Phosphate	—	3·2511	0·0195	**1·574**	2
Cupric oxide	—	3·0199	0·0121	10·1142	1
Basic carbonate (B.D.H.)	—	2·6281	0·0388	**0·6053**	1
,, (prepared)	—	2·6250	0·0064	98·3010	2
dl-valinate	—	2·5434	0·0067	41·6872	3
Glycinate	VII	2·5388	0·0162	32·1028	3
Cystinate	—	2·3824	0·0149	20·1614	1
Cyanide	—	2·1700	0·0111	—	—
Cuprous oxide (red)	—	2·3620	0·0085	4·5566	1
Oxalate	II	1·8295	0·0553	**0·4008**	1
Basic sulphate	—	1·7397	0·0009	153·2834	5
Phenylalaninate	—	1·5991	0·0197	**2·2775**	1
Basic chloride	VI, XV	1·5923	0·0034	13·2482	3
Tyrosinate	—	1·2145	—	—	—

* See p. 420.

Of the relatively insoluble derivatives tested, the **sulphide** ($\bar{b}=5{\cdot}2948$) yields the same regression coefficient as the soluble compounds but a lower LD 50 (series XIII, XX). Whether or not oxidation to cupric sulphate is a necessary step to toxic action cannot be deduced from the data. The **phosphate** ($\bar{b}=3{\cdot}2511$) is low in Table 4, but it was tested in three series (VI, XI, and XII) in all of which Bordeaux mixture yielded a relatively low

b value. These three direct comparisons indicate that the phosphate tested was not significantly inferior to Bordeaux mixture either in regression coefficient or in LD 50.

Bordeaux mixture. In its mean value, the regression coefficient for Bordeaux mixture is significantly lower than that of the cupric chloride. In direct comparisons the evidence of a difference is inconclusive; in series XVII the Bordeaux mixture value is lower, in series XIX*a* it equals, in series XIX*b* it exceeds that of the chloride; in series X no difference is shown between Bordeaux mixture and the sulphate. Further, as no consistent difference is shown in these series between the LD 50 for Bordeaux mixture, for sulphate, or for chloride, it may be concluded that there is no difference in their fungicidal value towards *Macrosporium sarcinaeforme*. Parker-Rhodes (1941) obtained a similar result, but the figures given by McCallan & Wilcoxon (1936) would indicate a wide difference between the LD 90's of Bordeaux mixture and of cupric sulphate to spores of the five other fungi they used.

The mean LD 50 to *M. sarcinaeforme* of all estimates for Bordeaux mixture (excluding that from series I) is antilog $\bar{3}\cdot599$ expressed as percentage copper in the spray applied. From the diameter of the area covered by the droplet, it follows that the maximum concentration of copper in the spore droplet at LD 50 is of the order of 2·3 p.p.m. The LD 50 of copper sulphate in series (*b*) is 2·7 p.p.m. copper. But as the solubility of Bordeaux mixture at laboratory temperature in water is of the order of 0·6 p.p.m. copper (0·2–0·3 p.p.m. according to McCallan & Wilcoxon, 1936) the spore suspension must accordingly bring the whole of the Bordeaux mixture copper into solution* at LD 50. Further, as Bordeaux mixture and cupric sulphate yield similar regression coefficients, it may be concluded that the spore intervenes in the toxic action of the sulphate or the chloride.

Burgundy mixture. As with the phosphate the low position in Table 4 of Burgundy mixture is misleading, for when the compound was compared directly with Bordeaux mixture (series XI) no difference in LD 50 or regression coefficient was obtained while, in series XII, the *b* value for Burgundy mixture is greater than that for Bordeaux mixture. It has frequently been suggested, particularly by Bedford & Pickering (1910, p. 28), that carbon dioxide is involved in the toxic action of the copper fungicides. On this hypothesis, it would follow that, as at least two stages of the interaction of carbon dioxide on the Bordeaux mixture precipitate have been accomplished in the preparation of Burgundy mixture, the latter would be expected to show a greater availability than Bordeaux mixture which would be shown, if not by a difference in regression coefficient, certainly by a difference in median lethal dose. The absence of any difference towards *M. sarcinaeforme* is an indication that carbon dioxide, whether atmospheric or from spore metabolism, plays no part in the process of toxic action of either Burgundy or Bordeaux mixtures.

The precise chemical character of the precipitates of these two mixtures is not yet established. The earlier view, that the Bordeaux mixture precipitate is a series of basic sulphates, was shown to be unlikely by Martin (1932), who presented evidence suggesting that the precipitate was cupric hydroxide stabilized by adsorbed calcium sulphate. The results of Wilcoxon & McCallan (1938) showed that, on weathering, the precipitate approaches the composition of hydrated copper oxide. In Burgundy mixture, Mond & Heberlein (1919) regarded the precipitate as a series of basic sulphates and a basic carbonate. The reactions of the spore to basic derivatives throws light on this subject.

It may be concluded that the responses of *Macrosporium* spores to normal cupric salts, Bordeaux and Burgundy mixtures are similar and that the mean regression coefficient displayed is associated with a common toxic ingredient of these sprays, the simplest of which is the cupric ion. On this hypothesis the regression coefficient given by compounds

* The possibility that the solution of the Bordeaux mixture precipitate is due to adsorption of copper by the spore and the consequent disturbance of solubility equilibria, is disproved by the solvent action of spore secretions, a subject to be discussed in a later paper.

furnishing cupric ions should be similar and of the order of 5·0–6·0. The **benzoate** (XXIV) ($b = 5{\cdot}1713$) and the **dinitrocresylate** (XXIII, XXIV) ($\bar{b} = 5{\cdot}4237$) conform to this rule, which may now be applied to the case of the oxides.

Cupric oxide in the hydrated form which results from continued leaching of freshly prepared hydroxide in the absence of carbon dioxide, gave in two tests (series XXVI, XXVII) a regression coefficient significantly lower than that of Bordeaux mixture though, in the second trial, there was evidence of a steeper line at higher concentrations. It is possible that this line is due to undecomposed Bordeaux mixture precipitate in traces too small to be detected by the sulphate reaction, but the inference of the low regression coefficient is that the mechanism of toxic action is more complex than the solution of the oxide by spore exudate.

Cuprous oxide in the more finely divided yellow form, gave the cupric ion slope in series XV but, in series XXVI and XXVII, gave lines of less steep slope. In the crystalline red form, cuprous oxide in all tests gave low values of the regression coefficient (series XV, XXIX), and, in trials XXVII and XXVIII, could be considered non-fungicidal. This result is in disagreement with Heuberger & Horsfall's results summarized in Table 3 and with those of Parker-Rhodes (1941) which permitted the inclusion of both red and yellow forms in his assessment of the variability of spores of *M. sarcinaeforme* to the cupric ion. The high χ^2 value of the mean regression coefficient probably arises through the fact that different preparations were used in each test because of the ready oxidation of pure cuprous oxide in aqueous suspension. But the difference in regression coefficients is a hint that the yellow and red forms differ in chemical character as well as physical properties. The observation by Parker-Rhodes (1941) that the LD 50 of cuprous oxide is twice that of copper in cupric form is confirmed with the yellow form (series XV, XXVII, XXVIII), which, if it be accepted that a further reduction cannot be achieved by greater subdivision of the oxide, would indicate that but half its copper is concerned in fungicidal action.

Basic compounds. The **basic sulphate** which, from its method of preparation, is the trioxysulphate $CuSO_4$, $3Cu(OH)_2$ and the **basic chloride** which, by analysis, is the corresponding trioxychloride, give low mean regression coefficients. Though the mean value for the sulphate is significantly greater than that of the chloride, the order is inverted in the direct comparison (series XV). The LD 50 of the basic sulphate shows wide fluctuations though, with the surprising exception of series XV, it is greater than that of Bordeaux mixture, while that of the basic chloride is consistently greater than Bordeaux mixture, approaching relative non-toxicity. This difference in LD 50 may be explained by the difference in method of preparation, for the basic chloride, being precipitated from boiling solution, will be of greater mean particle size than the basic sulphate precipitated at ordinary temperatures.

The **basic carbonate** (B.D.H.) yielded regression coefficients consistently less than those of Bordeaux mixture and greater than those of the basic sulphate whilst its LD 50 is normal. The **basic fluoride**, in those trials which permit the calculation of the regression coefficient (series IV, VIII, XX), yields a value below that of Bordeaux mixture yet which, from Table 4, appears to be greater than that of the other basic derivatives tested. The lower slope of the basic fluoride in the results of Fig. 1 appears to be that of the fluoride ion, for Parker-Rhodes (private comm.) found sodium fluoride insufficiently toxic to *M. sarcinaeforme* to permit the calculation of a regression coefficient which must accordingly be low.

The wide divergence between the regression coefficients yielded by the basic sulphate and Bordeaux mixture is strong support for the view that the latter is not a basic sulphate. Moreover, the reasoning put forward by Bedford & Pickering (1910, p. 22 et seq.), that, as the trioxysulphate yields a greater proportion of soluble copper by interaction with carbon dioxide than more basic sulphates, it should be a more effective fungicide than Bordeaux mixture does not conform to the present results. It should be pointed out, however, that the low regression coefficients obtained from the basic sulphate and the basic chloride, and the high LD 50 of the latter, do not necessarily mean that protective fungicides compounded from these derivatives will exhibit inferior field performances. Factors such as initial retention, coverage, and tenacity have to be taken into account in the assessment of protective value (Horsfall *et al.* 1937), and it has yet to be shown that the low regression coefficients of the basic sulphate and chloride are exhibited against fungi other than *M. sarcinaeforme*. In this connexion it may be mentioned that the two highest values of λ (the reciprocal of the regression coefficient) obtained by McCallan *et al.* (1941) were with the basic chloride on spores of *Botrytis cinerea* and *Alternaria solani*. No results are yet available for the zoospores of *Phytophthora infestans* for the control of which the copper fungicides are most widely used in this country. Nevertheless, it is reasonable to expect that, given non-intervention by the host-plant and equality in the other properties affecting field performance and in cost, protective fungicides made from compounds exhibiting higher regression coefficients than the basic sulphate and basic chloride would prove even more effective in the control of those fungal diseases in which the copper-sensitive spore is the stage attacked by the fungicide.

Modern ideas of the chemical constitution of the basic copper salts supply no reason for their low regression coefficients. It may, however, be noted that the theory of variability put forward by Parker-Rhodes (1942) requires that if a non-permeative compound is dissociated to a sufficiently small extent at the spore surface to n parts, one or more of which is permeative, the variability shown by the spore population to that compound will be n^2 times that shown towards the permeative part. But as the compounds under discussion exhibit a zero index of variation, variability will be inversely proportional to the square of the regression coefficient. Now the regression coefficients for basic sulphate, basic chloride, basic carbonate, basic fluoride and cupric sulphate are approximately in the ratio 1 : 1 : 2 : 2(?) : 4. But the constitutional formula of these compounds may be represented

$$[Cu\{(OH)_2Cu\}_3]\ SO_4 \text{ and } Cl_2,\quad [Cu\{(OH)_2Cu\}]CO_2 \text{ and } F_2, \text{ and } CuSO_4,$$

in the basic radicals of which copper appears in the atomic ratio 4 : 4 : 2 : 2 : 1; the inverse of the ratio of the regression coefficients.

If this argument is extended to Bordeaux and Burgundy mixtures, the regression coefficients of which are similar to that of copper sulphate, the inference is that the precipitates of these mixtures are not basic salts but, as already indicated in the case of Bordeaux mixture, stabilized cupric hydroxides. Chemical evidence would be required before it could be accepted that the Burgundy mixture precipitate is a stabilized cupric hydroxide, but Mond & Heberlein (1919) showed that the original precipitate is stabilized by adsorbed sodium carbonate from the change to the basic carbonate $[Cu\{(OH)_2Cu\}]CO_2$. It will be seen from Table 4 that the various samples of prepared basic carbonate gave a high χ^2 figure indicating a heterogeneity which may have been due more to the samples being at different stages in the change to basic carbonate than to different spore populations.

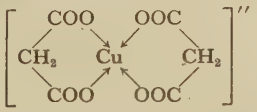
Copper derivatives in which co-ordination occurs

The solution of the Bordeaux mixture precipitate by the exudate of fungus spores has for long been regarded as a likely explanation of the origin of toxic copper concentrations and, in work to be described later, it has been shown that the mechanism of this solvent action is through complex ion formation. The examination of the fungicidal properties of complex copper compounds likely to be formed through the agency of the spore may, therefore, be expected to yield information on the nature of the process. Further, the relative toxicity of other complex derivatives in relation to their solubility in solutions of spore exudate or of known chemicals will provide data on the likely constituents of the spore exudate. For these reasons the complex compounds studied have included firstly, salts of the dibasic acids, hydroxy- and amino-acids, in which the cupric ion is subject in varying degrees to co-ordination with the anion, and secondly, various co-ordination

compounds in which co-ordination is independent of the anion, as in the cuprammonium series.

Salts of dibasic carboxylic acids. The oxalate, malonate, succinate and adipate are members of an homologous series of which the **oxalate** has the least fungicidal value ($\bar{b}=1{\cdot}8295$). The **malonate** ($\bar{b}=4{\cdot}4264$) approaches the toxicity of cupric sulphate in series X and (*a*), but is significantly inferior in series (*b*) and II against *Venturia inaequalis* in regression coefficient, and, in series (*b*), in LD 50 which, as the malonate is in solution, may be compared to that of the sulphate. The **succinate**, also relatively soluble, equals Bordeaux mixture in fungicidal properties in series II, XVIII and against *V. pirina* but is more toxic against *V. inaequalis* and, in series I and also in mean regression coefficient, against *Macrosporium sarcinaeforme*. The **adipate**, which is of lower solubility than the malonate or succinate yields a lower mean regression coefficient than the succinate but which differs little from that of Bordeaux mixture. This downward trend of toxicity shown by the adipate would suggest a peak of toxicity at the succinate were it not that the **sebacate**, a higher member of this homologous series, exhibits a regression coefficient ($\bar{b}=9{\cdot}9733$) greater than that of Bordeaux mixture. No reason for this is apparent unless it be related to the higher molecular weight of the molecule, for the **phthalate**, in which the $(CH_2)_8$ ring is replaced by the benzene ring, has also a high regression coefficient ($\bar{b}=7{\cdot}3114$) and is significantly better than Bordeaux mixture in LD 50 in series V and XXIV. This suggestion implies that the undissociated molecule is involved in the fungicidal action of the sebacate and phthalate.

In this series of salts the tendency to form complex ions is discussed by Riley (1929) and is greatest in the oxalate, decreasing as the number of CH_2 groups between the two carboxyl groups increases. In the discussion of the significance of a line of double slope previously described as cases (*b*) and (*c*), the explanation of two independent toxic constituents was suggested. Applying this hypothesis to the present results, it would follow that, in tests of the oxalate, the slope associated with the cupric ion does not appear but, with one exception, has been masked by the formation of complex ions characterized by a low regression coefficient. In series I (*Venturia inaequalis*) a hint of a line of steeper slope was obtained but in series II (*Macrosporium sarcinaeforme*), an anomalous result ignored in the calculation of the mean regression coefficient, the cupric ion slope predominates. With the malonate, however, the anomalous result (Table 4) is the low *b* of series (*b*) from a curve suggestion of double slope. This lower regression coefficient, which may be associated with the complex cuprimalonate perhaps of the type



also appears in the results obtained by Parker-Rhodes (1941) on the basis of which he included the malonate among the derivatives yielding complex ions. More frequently, however, conditions have permitted the cupric ion to exert its effect in yielding the higher *b* value found, for example, in series (*a*). As, in the succinate, the cupric ion appears to become the predominant toxic constituent, the general trend of the fungicidal observations is in agreement with Riley's observations on complex ion formation and with stereochemical considerations. Thus, when five- or six-membered ring formation is possible, as with the oxalate and malonate, a higher degree of complex ion formation would be expected. The possibilities of co-ordination by the carbonate anion to a four-membered ring are less and it is noteworthy that the biological results with Burgundy mixture yield no evidence of complex cupric ions.

Salts of hydroxy-acids. The **lactate** ($\bar{b}=5{\cdot}3801$) yields results so close to those of Bordeaux mixture (series X, XX) that there is no evidence that the cuprilactate (Wark, 1927) is involved in its toxic action. On the other hand, the **dl-malate** ($\bar{b}=5{\cdot}9426$) gave, in series

XVIII, XXII and XXIV, a regression coefficient significantly greater than that of Bordeaux mixture. This greater inherent toxicity can be ascribed to the cuprimalate ion but a direct test is frustrated: first, by the instability of the cuprimalate which like cupric malate, yields in solution cupric as well as cuprimalate ions; secondly, by the difficulty of obtaining evidence of double slope from lines both relatively steep. **Cupric cuprimalate** ($\bar{b}=5{\cdot}0712$) and **sodium cuprimalate** ($\bar{b}=5{\cdot}6157$) yield lines of slope similar to Bordeaux mixture (series XXII) and copper sulphate (series XXXIV). In the latter series in which a concentration ratio of $\sqrt{2}$ was used, there is marked evidence of double slope though the high χ^2 values are due more to scattering at individual concentrations. Thus the cupric malate results furnish a line of $b=7{\cdot}7262$ ($n=4$, $\chi^2=\mathbf{7{\cdot}1613}$) at high concentrations and one of $b=4{\cdot}4537$ ($n=11$, $\chi^2=38{\cdot}5027$) at the lower concentrations. The sodium cuprimalate results could likewise be expressed by a line of slope 3·4047 ($n=9$, $\chi^2=99{\cdot}0880$) at high concentrations and one of $b=6{\cdot}9593$ ($n=7$, $\chi^2=33{\cdot}6817$) at lower concentrations. In both cases the 't' test indicates a significant difference between the two slopes, the lower of which is not significantly different from that given by the sulphate in the same series, while the higher is of the order required for the cuprimalate ion. The importance of the cuprimalate in the fungicidal action of copper is also shown by the LD 50's of these three derivatives which are lower, in series XXX, than that of Bordeaux mixture and, in series XXXIV, than that of cupric sulphate. The fungicidal activity of cupric cuprimalate is so unlike that of the basic derivatives that no support can be given to the opinion of Pickering (1912) that this derivative is a basic salt.

The **tartrate** ($\bar{b}=4{\cdot}9563$) resembles Bordeaux mixture in toxicity (series II, III, XVIII, XXIV, XXX), but the low regression coefficient of series III, omitted from the calculation of the mean value though not different from that of Bordeaux mixture in the same series, prompted tests of **sodium cupritartrate** ($\bar{b}=4{\cdot}7871$). The similar behaviours of this compound, cupric tartrate and Bordeaux mixture indicate that the cupritartrate is equivalent to the cupric ion in fungicidal action on spores of *Macrosporium sarcinaeforme* and *Venturia pirina*, though the greater b value of the tartrate in the test against *V. inaequalis* suggests that this equivalence is not general.

The mean regression coefficient of the polyhydroxy-salt, **copper mucate** ($\bar{b}=3{\cdot}8952$), appears to be lower than those of other hydroxy-acid salts, an effect due to its use in series XI, XII in which that of Bordeaux mixture was abnormally low. By direct comparison, the mucate equals Bordeaux mixture in toxicity against the three fungi tested, though the low result of series VI is possibly an indication that, under the conditions of this test, a component of lower regression coefficient was involved.

Amino-acid derivatives. The simplest of the derivatives tested, the **glycinate**, yielded most variable results. When tested by direct addition to the spore suspension (series (*a*) and (*b*)), the mean regression coefficient is of the order of 2·0 with evidence of a second component of steeper slope. In trials on the spray deposit yielded by the glycinate solution, the higher value previously ascribed to the cupric ion was observed in series VII and IX, an intermediate value coupled with a high χ^2 value appeared in series X, while against *Venturia inaequalis* (series II) the value was again low. Parker-Rhodes (1941) also obtained a low b value for glycinate, which he ascribed to the complex ion, in tests on *Macrosporium sarcinaeforme* and on *Botrytis allii*. The graphical results on *B. paeoniae* given by McCallan & Wilcoxon (1936) yield a single line of slope approximately 1·7 when transferred to the

scale of probit germination-$\log_{10}$ copper concentration. In general, therefore, the complex ion is the predominant toxic constituent of the glycinate and, as its characteristic regression coefficient is much less than that of the cupric ion, it cannot be of greater inherent toxicity.

But, in an earlier paper (Horsfall *et al.* 1937) it was concluded from the examination of the graphical results of McCallan & Wilcoxon (1936) that the cupric ion is not wholly responsible for the fungicidal value of the copper-glycine complex and that there appears to exist in this solution a material of greater inherent toxicity than the cupric ion. The present evidence does not support the second of these conclusions, and it now seems probable that an explanation of the discrepancy is to be found in the fact that, as was pointed out in the earlier paper, whereas McCallan & Wilcoxon's results with the glycinate have yielded a linear probit germination-log concentration curve (i.e. $\alpha = 0$), those with cupric sulphate furnish a linear probit germination-concentration curve (i.e. $\alpha = 1$). The significance of this change in index of variation is not yet known but it can be induced, under certain conditions,

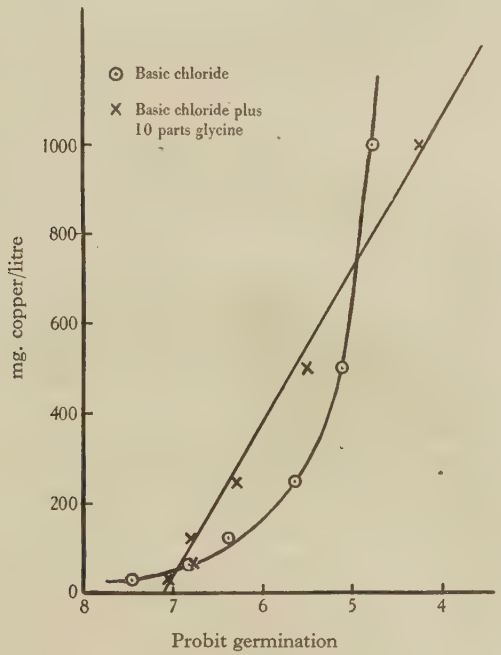


Fig. 2. The toxicity of basic copper chloride with and without glycine.

by the addition of extraneous substances. For example Fig. 2 is derived from tests on spores of *Macrosporium sarcinaeforme* of the basic chloride with and without added glycine. Without glycine the index of variation is zero; with glycine it is one. The similarity between this figure and that constructed from McCallan & Wilcoxon's results (Horsfall *et al.* 1937, Fig. 1) prompts the suggestion that, in the latter results, the chemistry of the spore droplet and the reactions of the spore are complicated by the presence of extraneous material. This material may be present not only in the 0·2% filtered orange juice added to stimulate germination of the spores of *Botrytis paeoniae*, the test organism in these trials, but also in the 0·1% ultra-filtered orange juice added to the spores of *Glomerella cingulata* which yielded unit index of variation with Bordeaux mixture, a point referred to on p. 424.

The next member of the homologous series of the amino acids, the **alaninate**, showed to a marked degree the same effect, on regression coefficient, of the technique of the test as was observed with the glycinate. When the diluted fungicide was added direct to the spore

suspension (series *a*, *b*) the mean *b* value was 1·5652; when the spore suspension was placed on the spray deposit, a mean value of 4·4773 resulted. In series IX the alaninate results coincide with those of the sulphate; in series X the high χ^2 value may be evidence of a second slope. It is, therefore, reasonable to associate the value 4·4773 with the presence of cupric ions, and that of 1·5652 with the copper co-ordination complex of alanine, which though not appearing in the sprayed slide tests of *Macrosporium sarcinaeforme* was observed in those of *Venturia inaequalis* and by Parker-Rhodes (1941) in tests on *M. sarcinaeforme*.

The **valinate** yielded a mean regression coefficient of 2·5434 which agrees with that (2·8324) given on the unsprayed slides. A component yielding a line of steeper slope is indicated by the high χ^2 value of the mean and appears in series IX. In Parker-Rhodes's (1941) results, however, the regression coefficient obtained with the valinate is similar to that of Bordeaux mixture, an indication that under the conditions of his tests this line of steeper slope predominates. The **leucinate** ($\bar{b}=3·2604$) gave straightforward results, in its two tests (series VII, IX).

Of the more complex amino-acid derivatives, the **tyrosinate** gave the extreme example of a line of double slope. In series VII the line of steeper slope ($b=5·0656$) predominates; in series IX that of less steep slope ($b=1·2145$). The former value has been associated with the cupric ion, the latter is then due to the co-ordinated copper tyrosine complex and has been taken as such in Table 4. It would be expected that, in solutions of the tyrosinate complex, the cupric ion will be in equilibrium with the complex ion and that, if one of the components is adsorbed by the spore, the equilibrium might be disturbed to an extent such that only the toxic effects associated with the adsorbed component would be observable. The evidence of lines of double slope disposes of this argument, and suggests either that time factors prevent the attainment of the equilibria involved or that the process is more complicated than cupric ion adsorption as would be the case if the cupric ion were not the penetrative toxic agent. But it would be expected that extraneous factors, in particular hydrogen-ion concentration, might influence ionic equilibria in solutions of the co-ordinated complex to a degree which would account for the wide divergence in the point of intersection of the two lines observed with the tyrosinate.

The **cystinate** ($\bar{b}=2·3824$) furnished slight evidence of a line of steeper slope in the high χ^2 value of the mean and in the case (*b*) curve of series VII while the **phenylalaninate** ($\bar{b}=1·5991$) yielded case (*b*) curve in both of its tests (series VII, IX).

There is, therefore, in these amino-acids, a greater tendency for the dominant constituent to be the co-ordinated complex as the series is ascended. On the electronic theory of valency, it is probable that the stability of the co-ordinated complex follows the same order at least in the series to the leucinate. By the same theory it would follow that the tendency to co-ordinate is far greater in the amino-acids than in the hydroxy-acids; in the present results the influence of co-ordination is more evident in the amino-acid than in the hydroxy-acid derivatives. Whether or not the respective *b* values of Table 4 can be accepted as the characteristic of the complex molecule or ion is doubtful but it is noteworthy that the values of the regression coefficient tend to fall as the series is ascended. It can be concluded that as these values are much less than that attributed to the cupric ion, the amino-acid derivatives play but an indirect part in the mechanism of the toxic action of copper. While they are probably responsible for the solution of copper from the relatively insoluble copper

fungicides, the results suggest, as was concluded by Parker-Rhodes (1941), that the complex derivatives thus formed require to be broken down before they are capable of exerting fungicidal action.

The **hippurate** (N-benzoylglycinate) was included in the trials because it was thought possible that hydrolysis might occur in the toxic reaction when the liberated glycine would be capable of co-ordination. Both the hippurate (series XVIII) and the benzoate (series XXIV) yield regression coefficients characteristic of the cupric ion and there is no evidence that co-ordination has affected fungicidal value. In series IV the hippurate exhibited a toxicity akin to that of the sebacate, an indication of greater inherent toxicity than Bordeaux mixture, again hinting, as with the sebacate, that the undissociated molecule is capable of a high degree of fungicidal activity.

Salts of two amino-dicarboxylic acids, aspartic (amino-succinic) and glutamic (amino-glutaric) can be compared, on the one hand, with the amino-acid derivatives and, on the other, with the salts of the dicarboxylic acids. The **aspartate** ($\bar{b}=3{\cdot}8228$) gave evidence of double slope in the test on *Venturia inaequalis* which may account for the slight discrepancies in the two tests on *Macrosporium sarcinaeforme* in which, in series III, the regression coefficient equals but, in series XVIII, it is less than those of Bordeaux mixture or succinate. By analogy it may be argued that the **glutamate** is also capable of furnishing lines of double slope, the lower of which is responsible for the aberrant value of series III omitted from the calculation of the mean ($\bar{b}=4{\cdot}5601$) in Table 4 and for the inferior results on *V. inaequalis*. In each case, the lines of lower slope may be attributed to co-ordination complexes, the existence of which, in dilute solutions of the two salts, was postulated by Pfeiffer & Werner (1937).

Cuprammonium and allied salts. The instability of cuprammonium sulphate and chloride precludes their examination by the technique used in these tests and the dinitrocresylate was studied despite the chance that in this salt the NH_3 groups may not be co-ordinated to the copper; the corresponding cupric salt was included to check the possibility that the acid radical possessed fungicidal properties. Although the mean value of the regression coefficient of **cuprammonium dinitrocresylate** is high, direct comparisons with cupric sulphate (series XVI) and with cupric chloride (series XVII) reveal no differences. Further, as the dinitrocresylate exerts a fungicidal action equal to that of Bordeaux mixture (series XIII, XIV), it may be concluded, first, that the cuprammonium ion, if present, is equivalent to the cupric ion in fungicidal activity and that, if the dinitrocresylate radical is fungicidal, this property does not appear within the range of concentrations of the regression line of the cupric ion.

Ethylenediaminocupric sulphate and **chloride** are stable enough to persist as spray deposits and, in direct comparisons with the corresponding cupric salts (series XXI), yielded significantly smaller b values of the order of 3·4. The solubility of the four compounds permits a comparison of their median lethal doses which being about nine times greater with the complex suggests, in conjunction with the evidence of the regression coefficient, that decomposition to cupric ion by spore excretions is a necessary step in the fungicidal action of the ethylenediaminocupric compounds. The results obtained with the **ethylenediaminocupric dinitrocresylate** and **ethylenediaminocupric bis-acetylacetone** bear examination from this point of view. The former (series XVI, XVII) has so high an LD_{50} that no indication of its regression coefficient was obtained. The latter

(series XXV, XXVII) has a regression coefficient and LD 50 of the same order as Bordeaux mixture.

An explanation of these results rests in the degree of dissociation of the complex in aqueous solutions or in the spore suspension. Thus both cupric and cuprammonium dinitrocresylates are decomposed by hydrogen sulphide whereas the ethylenediaminocupric solution yields no cupric sulphide. Similarly, in the presence of a dilute solution of sodium malate used to imitate a spore suspension, the cupric and cuprammonium derivatives give a yellow colour with sodium diethyldithiocarbamate, a reaction not given by the ethylenediaminocupric dinitrocresylate. The non-fungicidal character of the latter is therefore associated with high stability such as would be expected from the five-membered chelate groups. The electronic configuration of ethylene diaminocupric *bis*acetylacetone would similarly point to high stability, but, in dilute solution, this compound reacts with hydrogen sulphide or sodium diethyldithiocarbamate. The cupric ion concentration of dilute solutions is therefore the explanation of its fungicidal properties.

Summary

The germination of fungus spores exposed to known concentrations of different copper compounds was examined with the minimum interference by impurities such as nutrient material or leaf excretions. For this purpose, spore suspensions were either placed on the deposits obtained by drying sprayed cellulosed slides or admixed with solutions of the copper compounds on unsprayed slides. The fungi were chosen for high germination of spores in the absence of nutrient, the greater number of tests being made with spores of *Macrosporium sarcinaeforme*; spores of *Venturia inaequalis* or *V. pirina* were used in some parallel trials.

Under these experimental conditions linear relationships were obtained between the logarithm of the copper concentration and probit germination, the results falling in most trials on a single line enabling a characterization of fungicidal value by two statistics, one defining the slope of the line (the regression coefficient), another defining the position of the line (the median lethal dose). The regression coefficient affords a measure of the inherent toxicity of the fungicide and is, to a large degree, independent of the particle size of the material under examination. The median lethal dose affords a measure of the availability of the compound under test in so far as this property is determined by physical factors such as particle size.

Under certain conditions the probit-germination points fall on lines of different slope of which three cases may be distinguished:

(*a*) Germination at the lowest concentration is less than would be expected if the probit germination-dosage curve were a single line;

(*b*) Germination at the higher concentrations is less than would be expected for a single line;

(*c*) Germination at the lower concentrations is greater than would be expected for a single line.

Case (*a*) may be explained if a critical concentration has to be reached before the mortality-dosage curve becomes normally distributed (i.e. the Arndt-Schulz law). Case (*b*) and, possibly, case (*c*) may be explained by the presence, in the spore droplet, of two toxicants of dissimilar action. On these assumptions the results obtained indicate that:

(i) A wide group of copper compounds exhibit a common regression coefficient which can be attributed to the cupric ion. This group includes cupric chloride, dinitrocresylate,

phosphate, sulphanilate, sulphate and sulphide, cuprous oxide (yellow), Bordeaux and Burgundy mixtures.

(ii) Basic derivatives such as the basic sulphate, chloride, carbonate and fluoride yield regression coefficients smaller than that of cupric ion in the approximate ratio 1 : 1 : 2 : 2(?) : 4.

(iii) Derivatives in which co-ordination with the formation of complex ions can occur may exhibit more than one regression coefficient, a tendency which can be correlated with the stability of the complex ion. Thus of the salts of dibasic carboxylic acids, the oxalate usually, and the malonate sometimes, show regression coefficients lower than that of the cupric ion, whereas the succinate in all tests yielded a regression coefficient indistinguishable from that of the cupric ion. Of the amino-acid salts, the glycinate frequently yielded the cupric ion slope, the alaninate rarely, the valinate less rarely; the leucinate, however, yielded only a low regression coefficient which is general among complex amino-cupric derivatives, being given by the tyrosinate, phenylalaninate, cystinate, aspartate and glutamate.

The copper salts of the hydroxy-acids, lactic, malic, tartaric and mucic acids, generally yield regression coefficients of the same order as copper sulphate but, in the results with cupric malate, sodium and cupric cuprimalates, there is evidence of a component of steeper slope. When copper is co-ordinated to ethylenediamine a lower regression coefficient than that of the cupric ion is shown, the dinitrocresylate being so little toxic that evidence of its regression coefficient was not obtained. The copper derivative of ethylenediamino-*bis*acetylacetone exhibits the cupric ion regression coefficient. Cupric sebacate, phthalate and, in one test, the hippurate, yield regression lines steeper than that due to the cupric ion, an indication of high inherent toxicity which can be associated with the undissociated molecule.

In addition to ethylenediaminocupric dinitrocresylate, the basic arsenate, cupric ferrocyanide, quinaldinate, the copper salt of salicylaldoxime, cuprous iodide and thiocyanate may be classified as non-fungicidal to the spores tested. This non-toxicity is associated with the high degree of stability and insolubility of these compounds.

The simplest hypothesis concerning mode of toxic action which will account for the results obtained: (*a*) with Bordeaux and Burgundy mixtures and most relatively insoluble derivatives yielding the cupric ion regression coefficient, is solution by spore exudate by complex ion formation yielding cupric and complex ions of which the cuprimalate is that of most direct toxicity; (*b*) with soluble derivatives readily yielding cupric ions, is interaction with spore exudate, fungicidal action proceeding as in (*a*); (*c*) with yellow cuprous oxide, is a decomposition by which half the copper reaches the spore by the cupric ion route; (*d*) with red cuprous oxide, cupric oxide, basic copper compounds, and the complex amino-acid derivatives, is that fungicidal action follows a more complicated series of reactions the nature of which cannot be deduced from the results obtained; (*e*) with the sebacate and phthalate, is that the undissociated molecule may be directly fungicidal, an action independent of cupric ion formation.

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